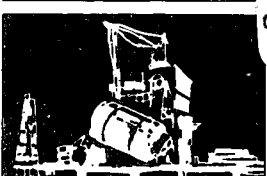
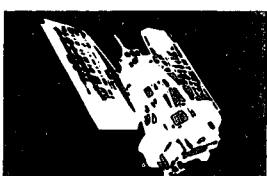
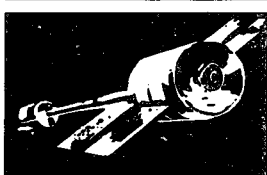
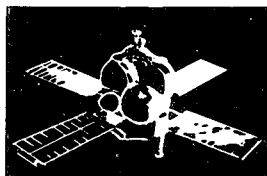
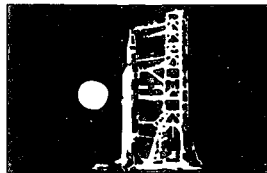
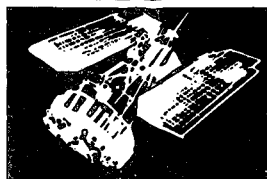


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IMBLMS PHASE B4

ADDITIONAL TASKS TASK 5.0

Microbial Identification System FINAL REPORT

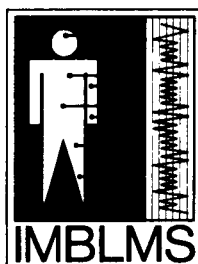
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FINAL REPORT

FOR

TASK 5.0

MICROBIAL IDENTIFICATION SYSTEM

IMBLMS PHASE B-4

ADDITIONAL TASKS

CONTRACT NAS9-10761

GENERAL ELECTRIC COMPANY

SPACE DIVISION

MICROBIAL IDENTIFICATION SYSTEM

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1.0 SUMMARY

A laboratory study was undertaken to provide simplified procedures leading to the presumptive identification (I/D) of defined micro-organisms on-board an orbiting spacecraft. Identifications were to be initiated by non-professional bacteriologists, i.e., crew members, on a contingency basis only. Key objectives/constraints for this investigation were as follows:

- o I/D procedures based on limited, defined diagnostic tests
- o Testing oriented about ten selected micro-organisms
- o Provide for definitive I/D key and procedures per selected organism
- o Define possible occurrences of false positives for the resulting I/D key by search of the appropriate literature.
- o Evaluation of the I/D key and procedures through a limited field trial on randomly selected subjects using the I/D key.

All objectives were met during the course of the investigation. In addition, a series of color photographs were taken of all significant reactions to each diagnostic test performed on the array of selected micro-organisms to provide supportive laboratory records and (potentially) serve as an aid during in-flight use.

Results of the investigation indicate that the defined tests are adequate for implementation of the I/D Key except for 4 of the 10 selected microorganisms (the Enterbacteriaceae) which cannot be identified unambiguously without the addition of one step for further biochemical tests, e.g., the Roche "Enterotube". The possibility of the occurrence of false positives was considered to be minimal with the significant exception of the same group cited above. Results of the field test using the I/D Key Nod Procedures was judged a qualified success due primarily to the limited number of subjects tested (13), and the decision (reflected in the Statement of Work) to omit fecal samples from the testing protocol.

Recommendations include:

- o The insertion of the Roche "Enterotube" into the array of diagnostic tools.
- o Emphasis on microscopic training as a critical skill in implementing the I/D key.
- o A study of the critical amount of cellular material required to eliminate false negative responses to the catalase test.
- o More extensive, additional testing of live subjects using I/D key procedures.

2.0 PROLOGUE

2.1 Problem Definition

Future manned spacecraft missions which call for extended flight times, i.e., more than two weeks, must be prepared for the necessity of dealing with possible microbiological problems. At the very least, scientists and medical observers on the ground will want a timely and representative sample from infections, rashes, etc., of suspected microbial origin. Optimally, the means to perform a presumptive identification of the possible causative agent should be available for on-board use. This also implies that spacecraft crew members, who are not likely to be trained microbiologists, will be called upon to perform these isolations/identifications. The approach to these problems suggests two basic questions, which in turn forms the foundation of this investigation.

1. What is the largest number of medically significant micro-organisms which can be presumptively identified with a minimum number of tests/procedures?
2. How can non-professional bacteriologists, i.e., crew members, be utilized to obtain meaningful samples and relevant information regarding incipient microbiological problems with a minimum amount of pre-flight training and in-flight time?

A presumptive answer to the first question was supplied by the Work Statement which defined ten micro-organisms and a set of diagnostic tests and/or tools. These organisms and diagnostic tests are listed in the following sections.

2.1.1 Selected Micro-organisms

a. Gram Positive Cocci (spheres)

1. Streptococcus pyogenes
2. Staphylococcus aureus
3. Diplococcus pneumoniae

b. Gram Negative Rods

Enterobacteriaceae

4. Salmonella typhi
5. Shigella dysenteriae
6. Proteus morganii
7. Enterbacter aerogenes
- - - - -
8. Pseudomonas aeruginosa
9. Haemophilus influenzae

Other

10. Candida albicans - fungus which stains strongly Gram positive

2.1.2 Diagnostic Tests/Tools

1. Blood Agar Plates - primary isolation of initial samples and visual observation of colonial morphology
2. Taxo Discs
 - o Type "A" for identification of beta-hemolytic strep
 - o Type "P" for differentiation of the pneumococci
3. Catalase Test - useful for biochemical separation of genera, especially strep and staph
4. Cytochrome - oxidase Test - used principally to separate Pseudomonas spp. from the Enterobacteriaceae
5. Gram stain - classical staining technique which, in conjunction with a microscope, divides the bacterial supp. into 4 broad categories on the basis of color uptake and cellular morphology (rod or sphere)
6. Antibiotic Sensi-discs
 - o Ampicillin - 10 mcg
 - o Gantrisin - 2 mg
 - o Furadantin/Macrodantin - 300 mcg
 - o Neomycin - 30 mcg
 - o Penicillin - 10 units
 - o Tetracycline - 30 mcg

2.2 Approach to the Problem

The attempt to answer the second question (2.1) forms the basic concept behind the approach to the problem and establishes the framework for the laboratory investigation described within this report. The exercise of each diagnostic test/procedure, in conjunction with the 10 selected organisms, is standard laboratory routine; the overall results of each test are obviously known in advance. Actual repetitive execution of these tests in the laboratory provides a better "feel", not only for the most efficient order of performance, but also for the objective criteria/reactions most likely to be meaningful or obvious to non-professional bacteriologists. The key to the success of the entire operation depends, ultimately, upon the extrapolation of the objective observations received from the spacecraft by professional bacteriologists (in Mission Control) who are equipped with the same I/D Key and Procedures as the crew member/operator. The end product of this trade-off can result in a reasonable assessment of how to meet each situation.

Therefore, the laboratory study was begun with the following major objectives:

2.2.1 Binomial Pathway for Presumptive I/D

Presumptive identification of each of the 10 selected organisms was to be organized along a "binomial pathway". At each decision point, a "yes" or "no" answer would lead logically to the next step until all 10 organisms were separated and/or identified. This goal is interdependent with the next two objectives.

2.2.2 Literature Search for Possible False Negatives

It was recognized that bacteria/fungus of medical significance, other than the 10 listed above, could be separated by the same diagnostic test procedures. What are these organisms, and how can their interference be identified or at least recognized? A search of method's text books, i.e. medical diagnosis, procedures manuals, etc. would be needed to uncover those organisms which can be associated with the occurrence of false positives.

2.2.3 Simplified "Cookbook" Procedures For Operator

Implementation of the presumptive I/D Key would be through a simple check list procedure. This will serve two purposes: (1) provide a training base for future operators, and (2) provide a basis for comparison by Mission Control experts to aid in this interpretation.

2.2.4 Color Photographs of Each Selected Organism versus Significant Diagnostic Tools

It was an objective to obtain color photographs of significant reactions to diagnostic tests performed on the selected micro-organisms, both to provide a permanent record supporting the findings, and potentially to serve as a common objective and for comparison between the on-board operator and Mission Control experts.

2.2.5 Evaluation of I/D Key by Live Testing of Proposed I/D Procedures

Once the I/D Key has been established and the corresponding procedures for its implementation have been written, an evaluation of the entire process will be undertaken. Due to budgetary limitations, it was decided at the outset that fecal samples would not be included in the testing procedure and that the number of random subjects tested would be limited to ten. Three samples: throat, nares, and skin, would be obtained from each subject and then subjected to the proposed I/D procedures for a total of 30 samples. Two obvious limitations were recognized:

- o Ten subjects represent a statistically marginal number with which to attempt an evaluation of the I/D key, and
- o Omission of fecal samples from each of the subjects precluded the possibility of the presumptive identification of Enterobacteriaceae (4 organisms) during testing.

However, it was felt that sufficient information could be gleaned from the exercise to obtain an objective assessment of procedural feasibility and false positives along with the limited presumptive identifications.

3.0 PREPARATORY LABORATORY EFFORT

Before the defined diagnostic tests could be applied to each of the 10 selected organisms, it was necessary to establish those cultures in an actively metabolizing state, or "biochemical maturity", in order that reproducible, predictable reactions may be expected to each test procedure.

Stock Cultures - Growth and Assay

The following organisms, designed by the Work Statement, were ordered from the American Type Culture Collection (ATCC)¹

<u>Micro-organism</u>	<u>ATCC #</u>
1. <u>Proteus morganii</u>	9237
2. <u>Enterobacter aerogenes</u>	13048
3. <u>Pseudomonas aeruginosa</u>	17423
4. <u>Streptococcus pyogenes</u>	14289
5. <u>Staphylococcus aureus</u>	6339
6. <u>Diplococcus pneumoniae</u>	9163
7. <u>Haemophilus influenzae</u>	8149
8. <u>Candida albicans</u>	10231
9. <u>Salmonella typhi</u>	6539
10. <u>Shigella dysenteriae</u>	13313

Procedures used in the rehydration and quantitative assay of the marker organisms are detailed in the Appendix, Sections 6.1 and 6.2. All cultures, except H. influenzae, were subcultured 6-8 times in either nutrient broth or Trypticase-Soy broth. Plate count assays were performed on the last 2 or 3 subcultures or until the titers appeared stable. H. influenzae demonstrated some reluctance to grow in submerged culture, and was carried by loop transfer on Blood Agar plates (Trypticase-Soy agar plus 5% sheep blood). H. influenzae subsequently produced growth in Brain Heart Infusion Broth plus Bacto-Supplement B (sterile yeast concentrate), but

¹ American Type Culture Collection 12301 Parklawn Drive, Rockville, Md. 20852

quantitative plate counts were not carried out. Table 3.1-1 summarizes the results of quantitative assays on 18 hour cultures of the final liquid passage.

As inferred from Table 3.1-1, the titer of each test organism, except H. influenzae, was considered satisfactory for initiation of the diagnostic testing. The cultures were considered "biochemically mature" i.e., likely to react in similar fashion as the naturally occurring organisms after isolation from human infections.

TABLE 3.1-1 RESULTS OF QUANTITATIVE PLATE COUNTS ON SELECTED
TEST ORGANISMS* FOLLOWING 18-Hr. GROWTH IN LIQUID
MEDIA AT 37°C

<u>Organism</u>	<u>Growth Medium</u>	<u>Passage Number</u>	<u>Titer</u>
<u>Strep. pyogenes</u>	Tryp. Soy Broth	8	1.9×10^8
<u>D. pneumoniae</u>	" " "	6	4.5×10^7
<u>C. albicans</u>	Nutrient Broth	7	1.4×10^6
<u>P. morganii</u>	" "	6	1.7×10^8
<u>Entero. aerogenes</u>	" "	6	4.0×10^8
<u>P. aeruginosa</u>	" "	6	6.8×10^8
<u>Staph. aureus</u>	" "	6	3.5×10^7
<u>Sal. typhi</u>	" "	6	4.0×10^7
<u>Sh. dysenteriae</u>	" "	6	1.8×10^7

* H. influenzae not assayed

4.0 RESULTS OF THE INVESTIGATIONS

4.1 Identification (I/D) Key

The routine diagnostic tools listed in Section 2.1 were tested in the laboratory against each organism. Details of the diagnostic test procedures are given in the Appendix, Section 6.3. The observed reactions were evaluated in terms of the most relevant and objective criteria, and in terms of the optimum order in which to perform each test. The end result of these evaluations is the I/D Key, shown in Figure 4.1-1.

As discussed in Section 2.2, the presumptive I/D Key follows a binominal pathway. This allows the operator to perform a series of objective tests, i.e., positive or negative; yes or no, which successively eliminates possibilities until a single organism, or group of organisms, can be presumptively identified. As implied in Figure 4.1-1, separation of most of the 10 selected micro-organisms from each other is straight-forward, with the exception of 3 genera of bacteria belonging to the family Enterobacteriaceae. Within the limitations imposed by the diagnostic tools defined for this study, no macro or microscopic examination or biochemical test is available for separating and identifying these genera. The separation/identification problem becomes even more acute when *Shigella* spp. other than *S. dysenteriae* are encountered; all other *Shigella* spp. are catalase positive. This holds true for the 5 major divisions, involving 11 genera of bacteria, which encompass the Enterobacteriaceae. This situation, together with the general consideration of false positives (with respect to the 10 selected organisms) is discussed in more detail in Section 4.3.

The Gram stain is an extremely important cog in the chain of tests used to separate these organisms and/or detect others with similar reactions (false positives). Standardized, i.e., consistent, Gram stain technique, therefore, is very important. Examination of Gram stained slides must be

done carefully and cautiously in order to avoid possible confusion or misinterpretation from artifacts. This is especially true with stains from possible fungus isolates where staining characteristics tend to vary as the age of the sample and which display a high incidence of pleomorphism as well. For these reasons, emphasis on microscopic training is strongly recommended.

The nasopharyngeal "Calgiswab" (thin)² was used to transfer single colonies of each organism to the Blood Agar (BA) isolation plate. Thus, all tests were originated from a single, isolated colony. The "Calgiswab" proved to be almost as easy to use and manipulate as the standard bacteriologist's loop. Some difficulty was experienced when colony separation was poor; i.e., adherence of cellular material from other colonies to the swab was difficult to prevent when colonies were too close together.

Satisfactory performance of the catalase test is also closely associated with the "Calgiswab". Positive test results depend upon the observation of small bubbles when 3% hydrogen peroxide is added to cellular material from the sample organism. Judgement as to the presence or absence of these bubbles depends directly on the amount of cellular material available, either on a strip of filter paper, or directly on the sample swab placed in a test tube containing H_2O_2 . Experience in this laboratory has indicated that critical amounts of test sample, i.e., cellular material from isolated colonies, is necessary to avoid possible false negative judgements when performing the test. This problem impinges directly upon the degree of colony separation demonstrated by the growth on the BA isolation plate. Therefore, it is recommended that some attention be directed toward defining, as objectively as possible, the amount of sample material required to produce an unambiguous result during performance of the catalase test.

2

Ordered from Colab Laboratories Inc., 3 Science Road, Glenwood, Ill. 60425. Stock Nos. 11-60-5 (thin); 11-20-1 (bulky).

On BA plates, the demonstration of hemolysis, either alpha or beta, is an observable phenomenon useful in the separation of some stains of bacteria. It should be reported that contrary to published literature, H. influenzae consistently demonstrated alpha hemolysis as well as sensitivity to Taxo P discs during the laboratory investigation phase. Technical personnel from ATCC, from whom the stain was obtained, could offer no explanation of this apparent anomaly. Diplococcus pneumoniae, another bacterial strain within the 10 selected organisms which normally exhibits alpha hemolysis and sensitivity to Taxo P can be separated by Gram staining: Gram negative rods and Gram positive cocci respectively. The Neomycin sensi-disc (30 mcg) is also quite useful in distinguishing H. influenzae from D. pneumoniae; H. influenzae is Neomycin sensitive.

4.2 Simplified Procedure for Use With I/D Key

In order to implement the I/D Key, a step-by-step procedure was prepared to aid the operator in performing the presumptive identification. The basic outline, or flow chart, upon which these procedures were constructed, is displayed in Figure 4.2-1. As shown, Taxo A and P discs and a Neomycin disc are deposited on the BA isolation plate as the first step. With the appearance of isolated colonies (following incubation), a number of options are available:

- o Determination of sensitivity to Taxo discs and/or Neomycin
- o **Macroscopic** examination of colonial appearance on the Blood Agar plate - see Table 4.2-1.
- o Performance of catalase and/or cytochrome-oxidase tests
- o Gram staining of suitable preparation followed by microscopic examination.

The decisions as to when to perform these tests is controlled by the bionomial system inherent within the I/D Key.

Macroscopic examination and description of colonia morphology is recommended as an integral part of this procedure, both as a valuable adjunct to the objective data normally collected, and as common reference point for additional amplifying questions from Mission Control scientists. Table 4.2-1 is suggested as a possible model for describing those characteristics which might be discussed during visual examination of the primary isolation plate.

An additional recommendation concerns the use of a visual aid in accomplishing the macroscopic examination. Employment of a low power magnification system, e.g., 4 or 5 x magnifying glass, or optimally, a laboratory stereoscope in conjunction with a maneuverable, variable intensity light source will substantially aid in providing higher quality information. Details which could be of critical importance frequently are not apparent without optical assistance.

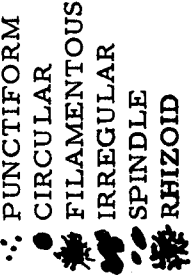
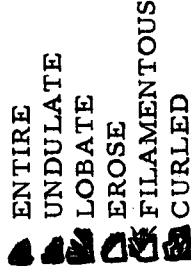
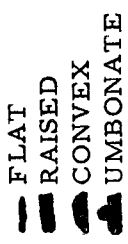
Subculture of single isolated colonies for determination of antibiotic resistance is made with the "Caligswab" (thin). By proper manipulation of the swab a contiguous lawn can be produced on a second BA plate. Placement of the antibiotic sensi-discs immediately following the transfer completes the task. Incubation for 18-24 hours will allow a roughly quantitative estimate of the degree of antibiotic resistance/sensitivity. Table 4.2-2 summarizes the results of 6 antibiotics at the stated concentration levels versus the 10 selected micro-organisms.

Figures 4.2-2, 3, 4 outline the suggested complete procedure to be followed in conjunction with the I/D Key (Figure 4.1-1). It should be emphasized that Figures 4.2-2, 3, 4 represent a procedure rather than instructions per se.

TABLE 4.2-1

SUMMARY TABLE - COLONY CHARACTERISTICS/DIAGNOSTIC TEST REACTIONS

ON TEN SELECTED BACTERIA

CHARACTERISTIC	DESCRIPTION	STREP. PY.	DIPL. PNEU.	CAN. ALBICANS	PRO. MORG.	ENT. AEROG.	PS. AERUG.	STAPH. AUREUS	SAL. TYPHI.	SHI. DYS.	HAEM. INFL.	REMARKS
FORM	 PUNCTIFORM CIRCULAR FILAMENTOUS IRREGULAR SPINDLE RHIZOID	X	X X	X X BLEND TOGETHER	X	X	X < 1 mm	X	X < 1 mm	X < 1 mm	X X	
MARGIN	 ENTIRE UNDULATE LOBATE EROSE FILAMENTOUS CURLED	X	X	X	X	X	X	X	X	X	X	
ELEVATION	 FLAT RAISED CONVEX UMBONATE	X	X	X	X	X X	X	X	X	X	X	GENERAL-MAGNIFICATION AT THE DISECTING SCOPE LEVEL IS PROBABLY NEED- ED FOR RELIABLE ASSES- MENT OF THIS CHARACTER- ISTIC (15-20 X)
OPTICAL CHARACTERS	OPAQUE TRANSLUCENT TRANSPARENT OPALESCENT IRIDESCENT	X	X	X	X	X WHITISH	X CENTER X EDGES	X OFF-WHITE	X WH-GREEN X EDGES X CENTER X EDGES	X	X	
OTHER	ODOR HEMOLYSIS COLOR	NONE BETA COLORLESS	NONE ALPHA GREYISH	NONE ---- GREYISH	STRONG STENCH GR.-WH.	YES --- WHITISH	GRAPE ODOR GREENISH	NONE LIMITED HEM. IM- MED. VIC. OF COL. WHITE	NONE --- GREYISH	NONE ---- WHITISH	NONE ALPHA? COLOR- LESS	
GRAM REACTION		G-POS COCCI CHAINS	G-POS. DIPLOCOCCUS CAPSULE	G-POS PLEOMOR.	G-NEG. ROD	G-NEG. ROD	G-NEG. ROD	G-POS COCCI CLUMPS	G-NEG. ROD		G-NEG ROD SHORT, FAT	
CATALASE		NEG.	NEG.	N/T	POS.	POS.	POS.	POS.	POS.	NEG.	N/T	N/T = NOT TESTABLE
OXIDASE		NEG.	NEG.	N/T	NEG.	NEG.	POS.	NEG.	NEG.	NEG.	POS. GENERALLY	
TAXO DISC A P		POS. NEG.	NEG. POS.	NEG. NEG.	NEG. NEG.	NEG. NEG.	NEG. NEG.	NEG. NEG.	NEG. NEG.	NEG. NEG.	NEG.	
SENSI-DISC N-30 (NEOMYCIN -30 MEG)		SENS.	INSENS.	INSENS.	SENS.	SENS.	SENS.	SENS.	SENS.	SENS. ±	SENS.	-15-

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE 4.2-2.

SUMMARY OF RESULTS OF REACTIONS OF SENSI-DISC TESTING OF SELECTED ORGANISMS

ORGANISM	AGENT/LEVEL					
	Grantrisin 2.0 mg G	Ampicillin 10 mg AM	Penicillin 10 units P	Tetracycline 30 mg Te	Macroclant 300 mg F/M	Neomycin 30 mg. N
<u>Streptococcus</u> <u>pyogenes</u>	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
<u>Diplococcus</u> <u>pneumoniae</u>	Sensitive	Sensitive	Sensitive	Sensitive	Intermediate	Intermediate
<u>Candida</u> <u>albicans</u>	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
<u>Proteus</u> <u>morganii</u>	Sensitive	Resistant	Resistant	Sensitive	Intermediate	Sensitive
<u>Enterobacter</u> <u>aerogenes</u>	Intermediate*	Intermediate	Resistant	Intermediate*	Sensitive	Sensitive
<u>Pseudomonas</u> <u>aeruginosa</u>	Intermediate*	Resistant	Resistant	Resistant	Resistant	Intermediate
<u>Staphylococcus</u> <u>aureus</u>	Intermediate*	Sensitive**	Sensitive**	Intermediate	Resistant	Sensitive***
<u>Salmonella</u> <u>typhi</u>	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
<u>Shigella</u> <u>dysenteriae</u>	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive
<u>Haemophilus</u> <u>influenzae</u>	Intermediate*	Resistant	Sensitive	Sensitive	Sensitive	Resistant

* Some Resistant Colonies

** Green Halo Around Zone

*** Halo at Edges

BASIC PROTOCOL FOR PRESUMPTIVE IDENTIFICATION OF ORGANISMS OF INTEREST

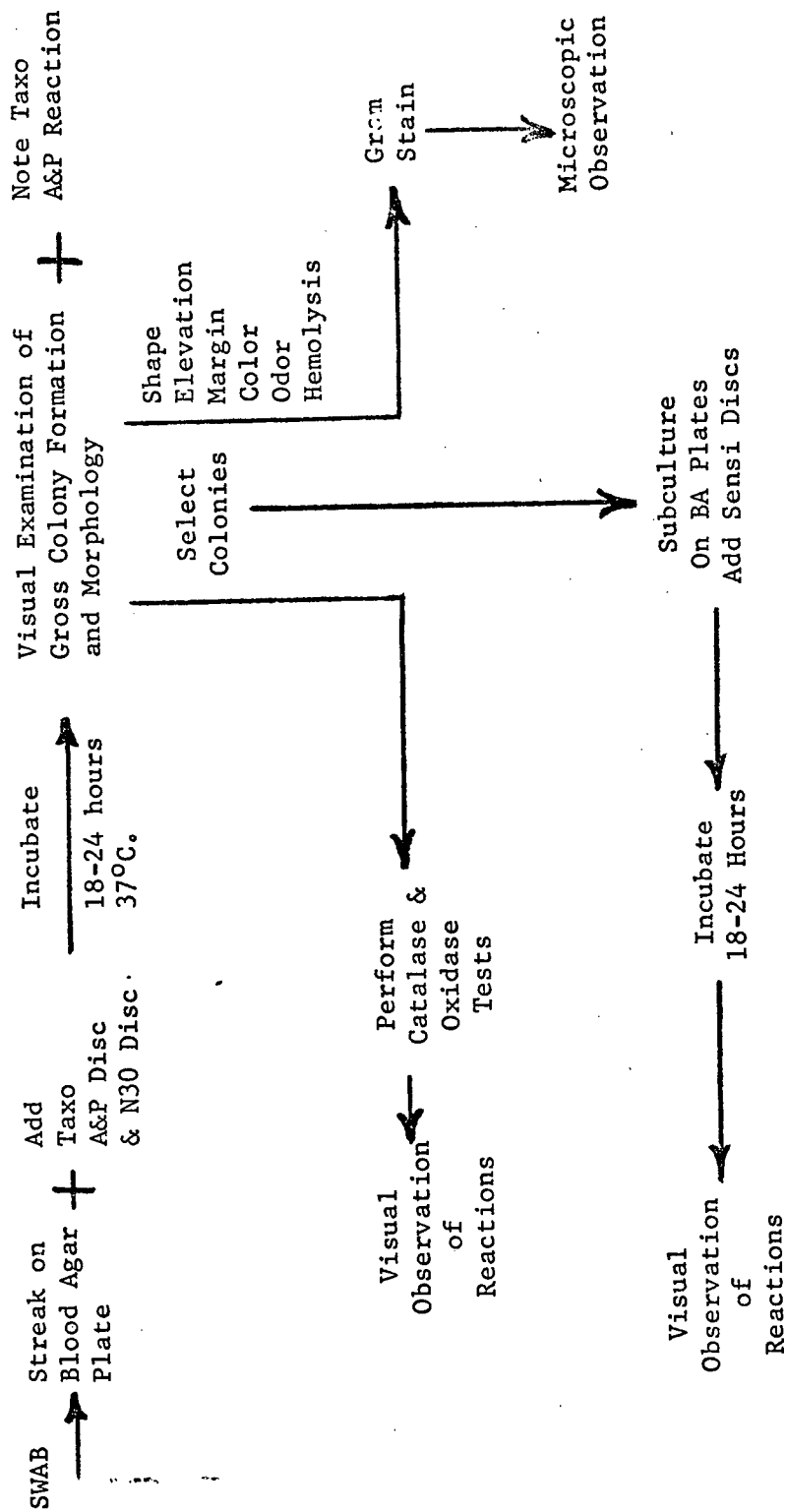


FIGURE 4.2-1

SIMPLIFIED PROCEDURES FOR USE OF I/D KEY

Figure 4.2-2 Phase I

1. Obtain swab sample.
2. Streak on upper portion of blood agar plate as shown:
3. Using 3 additional sterile swabs, make additional streaks as suggested by diagram:
4. Place Taxo A and P discs on opposite sides of a neomycin (30 MCG) sensi disc about 1 disc-diameter apart at the site of original streak:

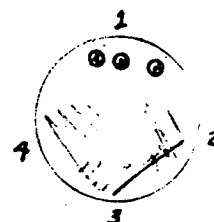
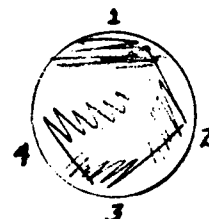
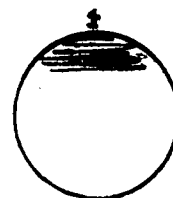
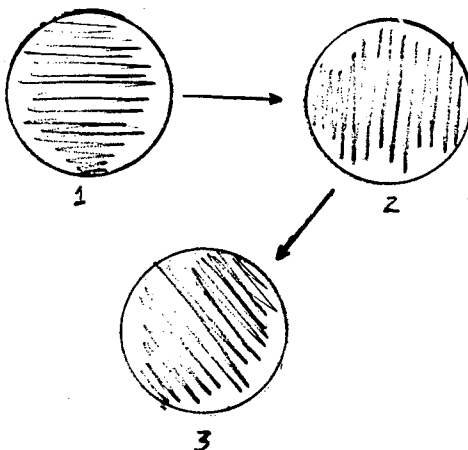


Figure 4.2-3 Phase II

1. Visually examine all incubated plates:
 - o using incident and transmitted light without magnification
 - o using incident light with magnification levels of 10-20X
2. Estimate total number of different colony types. Record characteristics of each type noted according to diagram on pictorial I/D key.
3. Note and record reaction of each colony type to the Taxo discs:
 - o sensitivity of either Taxo disc to any colony type - go to I/D key
 - o for colony types demonstrating no sensitivity - go to next step
4. Perform Catalase Test on selected isolated colonies - record results
5. Prepare and examine microscopically Gram Stain smear of each colony type - record findings.
6. Consult pictorial and flow I/D key for correlation of findings and/or additional tests, i.e. cytochrome oxidase test.

Figure 4.2-4 Phase III

1. Select colonies for antibiotic testing on basis of information derived from Phase II.
2. Use thin calcium alginate swab to carefully pick isolated colony from surface of primary isolation plate and spread on fresh bold agar plate according to the following procedure:



3. Apply sensi-discs and incubate at 37°C for 18-24 hours.
4. Examine incubated plates for presence/absence and/or degree of inhibition. Check each plate carefully to determine if different colony types exist, i.e., mixed culture.

4.3 Assessment of Possible False Positives by Literature Search Coorelation

Determination of possible interfering organisms, with respect to the 10 selected organisms, is of critical importance to the reliability of the proposed system (I/D Key). Other bacteria of medical importance may exhibit identical reactions to the array of diagnostic tests; therefore, information should be evaluated as to the estimated frequency and the kinds of different micro-organisms which could lead to incorrect, presumptive identifications.

In preparing this section, text books which emphasized procedural methods, i.e., "How-To", etc. were consulted, rather than papers from scientific journals. The text books employed are listed in alphabetical order at the end of this section as Table 4.3-2.

In addition, one assumption was made: Normal preflight procedures will include extensive microbiological analysis of the crew together with strong emphasis on cleanliness and decontamination efforts with respect to the spacecraft interior. If defined procedures were systematically and vigorously pursued, certain medically significant bacteria could be realistically ignored or eliminated from consideration as potential false positives, e.g., Bordetella pertussis or Listeria.

1. Streptococcus pyogenes

Sensitivity of the S. aureus to the Taxo A disc and Neomycin disc, plus rather pronounced beta (clear) hemolysis, make this organism unique within this identification scheme (I/D Key). Other organisms which may cause hemolysis on Blood Agar include: Listeria, various corynebacteria, hemolytic Staphylococcus, E. coli, and Pseudomonas. However, none of these other possibilities have been reported to demonstrate sensitivity to the Taxo A disc; they are also unambiguously separable by Gram staining. The Lancefield Grouping, of which S. pyogenes is designated as

to Group A, lists 3 additional groups of medical significance, i.e., "C", "G", and "D" (found most frequently in urinary infections), which also exhibit beta hemolysis. Again, sensitivity to the Taxo A disc identifies S. pyogenes (Group A) from the others.

The genus Streptococcus represents an ubiquitous group of bacteria found frequently both in the on man and animals. Other commonly isolated strains of Streptococcus include S. faecalis (enterococcus) commonly isolated from feces or urine, exhibits beta homolysis and is classified under Lancefield Group D (above). S. mitis formerly S. viridans, demonstrates alpha (greening) hemolysis and is considered a normal comensal in the oral cavity of most subjects. This species is not likely to cause interference with the I/D Key in separating S. pyogenes.

Estimate: High probability (90-100%) of identifying S. pyogenes within this system.

2. Diplococcus penumoniae

Sensitivity to the Taxo P disc and alpha hemolysis are solid clues to identity. Interference may arise from Haemophilus influenzae which is also Taxo P sensitive and exhibits alpha hemolysis. Gram staining is necessary to separate the two: D. pnemoniae is Gram positive with oval or spherical forms typically in pairs and encapsulated. H. influenza stains a short, fat Gram negative rod.

Estimate: Other stains of Diplococcus not known; if Gram stain is performed to eliminate H. influenzae; There is high probability of positive identification (90-95%).

3. Haemophilus influenzae

Discussion above on D. pneumoniae is applicable. There are 15 spp. of H. influenzae for the genus Haemophilus, but only about 6 are medically important or apt to appear. The Haemophilus stains are fastidious in their nutrient requirements (require x + v factors for growth), and variable in their response to Taxo P discs. Alpha hemolysis may or may not be exhibited which may cause confusion with the alpha hemolytic S. mitis; This is augmented by variable sensitivity to the Taxo P disc. Gram staining will be needed to unequivocally separate these two. Catalase is not testable, but most Haemophilus strains are cytochrome-oxidase positive. Bordetella pertussis also exhibits similar reactions and requires blood-rich medium to effect isolation; however, the possibility of encountering B. pertussis in this particular situation (spacecraft) has to be considered remote. The problem then becomes one of recognizing Haemophilus strains which may or may not exhibit hemolysis or demonstrate sensitivity to Taxo P discs, primarily through Gram staining. Also, H. influenzae is sensitive to Neomycin.

Estimate: Possibility 60-70% of arriving at presumptive identification of H. influenzae.

4. Staphylococcus aureus

Staphylococcus is separable from Streptococcus primarily through the catalase test (Straph is positive). However, objective separation of Staph. aureus from S. epidermidis, S. saprophyticus and Micrococci spp. with the limited array of test available in the I/D Key is probably not possible. Testable biochemical differences exist; separations are also possible with anaerobic incubation, but neither are available within the I/D Key System. S. aureus does display a smooth, shiny, milky-white or yellow colony on BA, and after approximately 20 hours the colonies tend to exhibit a small ring of beta hemolysis around the periphery.

Gram stain characteristics between S. aureus and Micrococci can be significant: Micrococci normally display Gram positive spheres in pairs, fours or small clusters, with the cocci generally being more uniform in size. Straphylococci, on the other hand, tend to occur as Gram positive spheres in large clusters with the cells showing great variation both in size and stain-retaining power.

There is the possibility of confusion of S. aureus with Aerococci which can show variable reactions toward the catalase test (Aerococci can be feebly positive or frankly negative). Aerococci also demonstrate alpha hemolysis. However, the possibility of encountering Aerococcus is considered remote.

Estimate: The site of the sample will probably furnish the best indication for separation of S. aureus from S. Epidermidis. Separation of S. aureus from the Micrococci is dependent largely upon the operator's training, experience, and judgement. Identification of S. aureus: 75-85%

5. Candida albicans

Separation of Candida albicans from bacterial specimens by visual observation alone will require judgement. Candida can be isolated from almost any body site and generally exhibits a smooth, pasty colony on the BA. This colonial morphology is in contrast to other fungi which generally show a diffuse, filamentous growth. The organism most likely to appear which can be confused with Candida is Geotrichosis candidum which can be isolated from a variety of infected material. G. candidum colonies are membranous, mealy, flat and cream to white in color; this color and morphology is similar to C. albicans. Gram staining is definitely needed to separate C. albicans from G. candidum: Under low power, Candida exhibits pseudomycelium with chlamydospores. G. candidum will exhibit rectangular arthrospores and true, branching hyphae. C. albicans also exhibits complete resistance to all the antibiotic sensi-discs employed in this I/D Key.

Estimate: With a little experience, Candida albicans should be presumptively identified 90% of the time it occurs.

6. Shigella dysenteriae

S. dysenteriae is a member of the family Enterobacteriaceae, but is the only Shigella species which is catalase negative, making it a unique separation. The most likely organism which might be confused with S. dysenteriae is Actinobacillus (Glanders). However, Actinobacillus grows very slowly, and although it is a Gram negative rod, it tends to stain very irregularly and display a high incidence of pleomorphism. On the other hand, S. dysenteriae displays strongly Gram negative, large fat rods. Actinobacillus is judged an unlikely candidate to be isolated. Separation of other strains of Shigella (catalase positive) are discussed in a following paragraph.

Estimate: Preumtion identification of S. dysenteriae is considered 90-100%.

7. Pseudomonas aeruginosa

P. aeruginosa, if it is in sufficient numbers on the isolation plate, can often be recognized by a characteristic grape-like odor and a green pigment exhibited by the colonies on the agar. P. aeruginosa is catalase positive and Gram negative, displaying large, fat rods to long, slender, almost filamentous rods. Separation from the Enterobacteriaceae is by the cytochrome-oxidase test which is negative for Enterobacteriaceae.

Estimate: Estimate 90-100% possibility for presumptive identification within I/D Key.

8. Enterobacter aerogenes

9. Proteres morganii

10. Salmonella typhi

The three organisms listed above are also members of the Enterobacteriaceae family. This represents a rather large division which is divided into

5 major groups, which in turn, encompass a minimum of 17 identifiable strains of enteric bacteria. Table 4.3-2 describes these subdivisions and the strains involved. In addition, Actinobacillus, Pasteurella, and Chromobacterium are possible interfering genera, although the probability of isolating these organisms under spaceflight conditions is judged unlikely. The major problem lies with the separation among the Enterobacteriaceae, even within the 5 major subdivisions. No test or procedure is available within the currently structured I/D Key that will objectively separate these groups. Expert bacteriologists might make venture educated guesses as to genera, based on the sample site, colonial morphology and gram stain cellular structure, but it is unreasonable to expect amateur microbiologists to attempt such subjective identifications.

It is recommended that the Roche "Enterotube" be considered for inclusion within the diagnostic tests currently defined by the I/D Key. The device consists of an 8-chambered tube containing selected, sterile agar media. A sterile, protected rod, which runs down the middle of each chamber, is accessible through removable end-caps. To use, one end of the rod is touched to a single colony on an isolation plate, and the rod is withdrawn through the chambers, successively inoculating each chamber. The final chamber contains a positive control which assures inoculation. After incubation, color changes in each chamber are noted and the organism is identified by consulting a reaction chart and a table in which all possible permutations are displayed. (See Appendix, Section 6.5)

Estimate: Without additional test capability, there is virtually no possibility of presumptively identifying any of the Enterobacteriaceae.

TABLE 4.3-1

BIBLIOGRAPHY - SOURCES CONSULTED IN ASSESSMENT OF FALSE POSITIVES

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- Gradwohl's Clinical Laboratory Methods and Diagnosis, Volume 1, 6th Edition. Edited by Frankel, S., Reitman, S., and Sonnenwirth, A.; C. V. Mosby Company, Saint Louis, 1963.
- Pelczar, M. I.: Manual of Microbiological Methods by the S.A.M. Committee on Bacteriologic Technic, McGraw-Hill Book Co., Inc., New York, London, Toronto, 1957.
- Rohde, Paul A., Editor, BBL Manual of Products and Laboratory Procedures, 5th Edition, copyright 1958, 3rd reprinting 1970. BBL, Div. of Becton, Dickinson and Co., Cockeysville, Maryland 21030.
- Schneierson, S. S.: Atlas of Diagnostic Microbiology, Published by Abbott Laboratories, North Chicago, Ill., 1965.
- Skerman, V.B.D., A Guide to the Identification of the Genera of Bacteria, 2nd Edition, The Williams & Wilkins Co., Baltimore, 1967.

Table 4.3-2

*Group Differentiation of Gram-Negative Organisms (Enterobacteriaceae)

	<u>Group</u>	<u>Genera</u>	<u>Strains</u>
1.	Escherichia Shigella	Escherichia Shigella	
2.	Proteus Providencia	Proteus Providencia	Vulgarie Mirabillis Morgonii Rettgeri
3.	Klebsiella Enterobacter Serratia	Klebsiella Enterobacter Serratia	Aerogenes Aerogenes Hafniae Liquefaciens
4.	Salmonella Arizona Citrobacter	Salmonella Arizona Citrobacter	
5.	Edwardsiella	Edwardsiella	

*Roche Diagnostics, Division of Hoffman-LaRoche , Inc.
Nutley, New Jersey 07001

4.4 RESULTS OF LIVE SUBJECT TESTING USING I/D KEY

Evaluation of the I/D Key can only be accomplished by applying those tests and procedures to actual subjects. The question of how many subjects and what sample sites is addressed in the Statement of Work: A total of 10 subjects will be sampled from each of 3 sites, i.e., throat, nose, and skin (forearm), for a total of 30 samples to be subjected to analysis (presumptive identification) according to the scheme derived from the I/D Key. Omission of fecal samples obviated the possibility of uncovering 4 of the selected organisms (the Enterobacteriaceae), i.e., Proteus morganii, Shigella dysenteriae, Salmonella typhi, and Enterobacter aerogenes. Although the total number of subjects is small, statistically, it was felt that sufficient information could be extracted to derive meaningful and useful information about the Key.

4.4.1 TEST PROTOCOL

During actual testing, thirteen subjects were selected at random from the GE-Valley Forge STC population. Attempts were made, through the in-house Health Service, to obtain subjects suffering from obvious, upper-respiratory infections. Due probably to the time of the year (late summer), and the relatively short period devoted to actual testing (3 weeks), it was not possible to find such subjects, other than those suffering from obvious allergic-type reactions.

Three Caligswabs³ (bulky) were used to collect samples from each subject; soft pallet area in the throat (dry swab); nares (swab moistened in sterile Nutrient broth); and skin of forearm (moistened swab). Each swab was streaked on the upper quarter of a Blood Agar (BA) isolation plate. Using 3 additional, sterile swabs, a "plate dilution" was effected. Immediately thereafter, a Taxo A, a Neomycin (30 mcg) disc, and a Taxo P disc were placed over the area of the original streak

³ See Footnote #2

(heaviest concentration). This procedure is identical to that outlined in Figure 4.2-2, 3, 4: Simplified Procedures for Use of I/D Key. Incubation was at 37°C for 22-24 hours.

The information resulting from each swab sample/BA plate was recorded on a form devised for the testing. An example of this form is shown as Figure 4.5-1.

The raw data collected in reproduced in the Appendix, Section 6.4.

4.4.2 RESULTS OF LIVE SUBJECT TESTING

Results of the testing of 13 subject from 3 sample sites are summarized on Table 4.5-1.

As shown in the Table, the two most numerous genera isolated were staphylococcus and non-pathogenic streptococcus. Differentiation between staph and strep was easily accomplished by use of the catalase test (staph is catalase positive). However, differentiation between staph and micrococcus spp. or strep and areococcus spp. was not possible, nor was accurate separation possible within the strep. spp. itself except possibly for S. mitis which was identifiable by virtue of the alpha homolysis and non-sensitivity to Taxo-A discs. S. mitis was identified in every throat culture of the 13 subjects.

Candida albicans was tentatively identified from the Gram stain microscopic examination and comparison with the photo-micrographs which accompany the I/D Key.

Identification of the Corynebacterium spp. was accomplished on the basis of colonial morphology and Gram stain examination which displayed typical palisading arrangements among the club-shaped, intensely Gram positive rods. This differentiation is not a part of the system I/D capability.

There was a total of 4 organisms which were essentially untypable through employment of the diagnostic tests available with the I/D Key. No other attempt was made to identify these isolates by use of standard sugar fermentation tests, typing Sera, etc.

In addition, the following general observations can be made concerning the live subject testing:

1. The calcium Alginate Swabs, both the bulky and thin varieties, are excellent substitutes for the microbiologists's loop.
2. The swabs become less than optimum when good physical separation of the different colony types has not occurred as a reoccurrence is the result of variable which are essentially uncontrollable, and therefore, not amendable to corrective suggestions.
3. The technique suggested by the I/D Key is simple and straight-forward. The primary emphasis during the construction of this key was to provide for a series of binomial steps which would lead, eventually, to a logical, unambiguous result, i.e., a presumptive identification. Experience to date indicates that this circumstance cannot be expressed in every instance.
4. The deficiencies alluded to above can be overcome to some extent by sufficient skill on the part of the operator, i.e., training; or by adding one or two additional diagnostic tests to the basic armamentarium; optimally, both.
5. The catalase test is one of the more useful tests employed. It appears that with some strains likely to be encountered, e.g., *Micrococcus* spp., *Aerococcus* spp., the amount of cellular material gathered for this test may be critical. The difference between a true positive and a false negative may be dependent upon a critical mass necessary to confirm by visual examination the gas bubbles produced by the addition of the hydrogen peroxide.

It was concluded that the live subject testing was not structured in sufficient depth to answer statistically the degree of confidence with which the 10 selected organisms could be identified presumptively during random testing. Never the less, the exercise did provide invaluable information on the technique, and their applicability by non-professional micro-biologists. Such a preliminary test would have been required before embarking on a large scale testing exercise.

MICROBIAL IDENTIFICATION

CODE NUMBER:		
SUBJECT NAME:		
SITE:		
SWAB:	MOIST -	DRY -
	MEDIUM -	
DATE:		TIME:

PRIMARY CULTURE INFORMATION	
RESULTS ON BLOOD AGAR (18-24 HOURS)	
TAXO DISC REACTION:	
TAXO A - PRESENT	ABSENT
TAXO P - PRESENT	ABSENT
NUMBER OF APPARENT COLONY TYPES:	
AROMA (IF PRESENT)	
DESCRIPTION:	
OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)	

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G
				FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.	HEMOL.

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Comma
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores
- C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle
- D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Grose
5. Filamentous
6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	

- E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opalescent
4. Irridescent
- G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

FIGURE 4.5-1

TABLE 4.5-1

SUMMARY OF LIVE SUBJECT TESTING USING I/D KEY

ORGANISM-PRESUMPTIVE IDENTIFICATION	SAMPLE-SITES		
	THROAT	NARES	SKIN
Strep/Aerococcus	29	5	5
Staph/Micrococcus	8	18	11
Candida	0	1	0
Other			
Corynebacterium	1	4	2
Unknown	<u>2</u>	<u>4</u>	<u>0</u>
Total	40	30	18

5.0 CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Results of the experimental laboratory work and literature analysis on the simplified identification scheme indicate adoption of the following conclusions:

- o The test and procedures defined for the study are satisfactory for implementation of the I/D Key concept for 4 of the selected organisms (Enterobacteriaceae).
- o The possibility of the occurrence of false positives is minimal with the significant exception of the 4 organisms cited above.
- o The basic design concept of the simplified presumptive identification is sound.
- o Evaluation of the I/D Key and procedures with limited, live subject testing has demonstrated the value and applicability of the procedural tools and techniques.

RECOMMENDATIONS

Mindful of the preceeding conclusions, the following recommendations are made:

- o Insertion of the Roche "Enterotube" into the array of diagnostic tools and procedures to effect separation and presumptive identification of the family, Enterobacteriaceae (see Appendix, Section 6.5).
- o Use of some modest form of optical magnification (2x-5x) to aid the on-board operator to describe and define more accurately the gross morphological characteristics for real time ground information.
- o Emphasis on microscopic training and technique as a critical skill in implementing the I/D Key.
- o A study of the critical amount of cellular material necessary to perform the Catalase test, i.e., eliminate possibility of false negatives.
- o Investigate a statistically validated field trial for a more extensive evaluation of the I/D Key and procedures.

SECTION 6

APPENDIX - PROTOCOLS AND RAW DATA

6.1 REHYDRATION OF STOCK CULTURES FROM ATCC

Bacterial and fungal cultures from the American Type Culture Collection (ATCC) are routinely sent through the mail in the lyophilized state. The dried material is encased in a glass vial protected by a cotton plug. This vial is encased in a larger flame-sealed glass capsule with asbestos wadding between the cotton plugged inner vial and the larger, protective vial. The entire assembly is wrapped in cotton wadding and packed inside a double shipping container. Thus, the likelihood of breakage is slight and, if there is, the danger of possible infection is greatly reduced.

To open the vial and rehydrate the culture for experimental use, the following equipment is suggested:

1. Bunsen-type flame
2. Container of water (sterile condition not required) and pipette
3. Tubes containing sterile bacteriological broth, i.e., Nutrient broth, Tryp-Soy broth, etc. Amount of material is dependent upon number of cultures to be rehydrated - 0.5 ml/culture.
4. Sterile pipettes
5. Sterile forceps
6. Standard bacteriologist's loop
7. Tubes, poured plates, agar slants, etc. of prepared media - depending on micro-organisms to be cultured.

Procedure

1. Carefully heat the flame-sealed end of the outer protective vial in the Bunsen flame. The asbestos wadding protects the smaller vial containing the lyophilized culture from heat.

2. Allow a few drops of water to dribble on the heated end of the glass. This will cause the glass to crack and allow access to the inner vial.
3. With the sterile forceps, carefully remove the inner vial.
4. Loosen the cotton plug in the culture vial.
5. Remove the cotton plug and introduce 0.5 ml of appropriate sterile broth (sterile, distilled water may also be employed) and allow dried material to rehydrate. The tip of the sterile pipette used to introduce the broth may be used to mix the solution. The time required to solubilize the dried material varies with the nature of the organism, the original suspension-liquid, and the efficiency of the lyophilization process.
6. Distribute portions of the rehydrated material into appropriate culture tubes, etc. for incubation.

6.2 ROUTINE PLATE COUNT ASSAY OF STOCK CULTURES

It was considered essential that each test organism demonstrate a stable, viable count following constant incubation conditions, i.e., 18 hours at 37°C before subjecting them to any of the defined tests or procedures. Consequently, plate count assays were performed on successive 18 hour cultures of each organism, except H. influenzae⁴, until stable titers were obtained. It was considered that this condition represented a state of "metabolic maturity" for each micro-organism, and thus would exhibit reproduceable reactions to the standard tests/procedures for the I/D Key.

The following procedures were pursued during standard pour-plate determinations:

1. Screw-cap culture tubes (20 x 150 mm) were employed as growth tubes, containing 9 ml each of either Typticase-Soys broth or Nutrient broth (see Table 3.1-1 in text).
2. Each tube was inoculated with one drop of the previous 18 hour culture which had been stored at 4°C until needed.
3. Tubes were incubated at 37°C for 18 hours with screw caps loose, i.e., not screwed down.
4. Mixing of each culture tube was accomplished in the following steps:
 - a. Agitation for 30 seconds in a mechanical mixer, e.g., Vortex Jr.
 - b. Additional agitation for 2 minutes in a Bronson "Sonigen" ultra-sonic generator, filled to predetermined mark with a solution of 0.3% Tween 80 in distilled water. The tube was inserted in the bath so that the level of fluid in the tube equaled the level in the bath. The generator was tuned by ear to the highest discernable pitch.
5. Each tube was serially diluted in 10-fold steps using 9 ml blanks of either Tryp-Soy or Nutrient broth. Mechanical agitation was employed between each dilution.
6. The appropriate dilution tube was used to provide triplicate samples of 0.1 ml each, i.e., 10⁵ diln. $\xrightarrow{0.1 \text{ ml}}$ 10⁶ plate. Three dilutions per sample were plated.

⁴H. influenzae was cultured by successive transfers on Blood Agar plates.

7. Melted agar, held at 45°C in a water bath, was poured into each of the sample plates and swirled to mix the agar medium. The same nutrient agar base was employed for the plate medium as that used for 18 hour growth medium and for the dilution fluid.
8. Plates were allowed to solidify, inverted, and placed at 37°C for 48 hours. Plates were counted with the aid of a New Brunswick colony counter.

6.3 DIAGNOSTIC TEST PROCEDURES

Taxo Discs

Taxo A and P discs were employed in connection with the Neomycin (30 mcg) disc.. Each disc was placed in the upper quarter of the isolation plate (Blood Agar Plate) in the area of the initial spreading of the sample (heaviest concentration). Discs were always placed about 1 disc-diameter apart in order to provide sufficient separation of possible areas of inhibition, and to allow space for individual concentration gradients resulting from each disc. No effort was made to consider possible effects of disc placement during a zero gravity environment, although the problem of dispensing these discs during weightless operation appears to present no serious technical problems.

Antibiotic Sensi-Discs

The discussion above is applicable to the sensi-discs. The application of the discs singly or simultaneously during zero gravity operations is a detail which can be addressed at a latter date.

Catalase Test

Two methods were used to test for the presence or absence of Catalase: filter paper technique and test tube assay.

1. Filter Paper

A thin "Calgiswab" was used to obtain cellular material from an isolated colony on the Blood Agar isolation plate. The swab was then rubbed across the surface of a strip of filter paper (approximately 10 x 75 mm). Either a syringe or a pipette was used to apply small amounts (0.2 - 0.4 ml) of 3% hydrogen peroxide (H_2O_2) directly to the filter paper. The immediate expression of bubbles on the surface of the filter paper indicated the

presence of Catalase. Performed in this manner, the test is directly applicable to zero gravity operation.

Comment - Continued experience with the test suggested that absolute discernment of the presence or absence of bubbles was not always possible. Indications were that the amount of cellular material deposited on the filter paper was critical. The Catalase test is an extremely useful test, particularly in the separation of Staph and Strep colonies. Therefore, a modest study to define, within some acceptable limits, the amount of cellular material required for reliable test results, i.e., production of easily visible bubbles, is indicated.

2. Test Tube Assay

In this variation, the "Calgiswab" containing the sample was inserted directly into a test tube (13 mm) containing approximately 0.5 ml of 3% H₂O₂. The expression of small bubbles from the vicinity of the cellular material was easily visible through the test tube. Some equipment modification would be necessary to employ this technique during zero gravity operation.

Comment - Care should be taken in this assay to differentiate between air bubbles trapped within the fibers of the swab and those bubbles which issue as a result of the catalase reaction. The former tend to be larger and few in number, while the latter are very small in size and quite rapidly expressed.

Cytochrome - Oxidase Test (Patho-Tec Strip)

Cellular material was obtained on a "Calgiswab" in the same manner as defined for the Catalase test and applied to the reagent end of the Patho-Tec Strip. With a

positive test, i.e., cytochrome-oxidase present, the reagent zones turn a bright blue color within 30 seconds; a negative result exhibits no change in color. Weakly positive reactions can occur where a pale blue color will develop. Addition of a few drops of water to the Patho-Tec Strip makes no discernable difference to the final results.

Gram Stain Technique

The Gram stain procedure employed in this study followed the technique as defined by the Hucker modification⁵. The procedure is as follows:

- | | |
|---------------------------|-------------|
| o Gentian Violet Solution | 1 minute |
| o Gram's Iodine | 1.5 minutes |
| o 95% Ethyl Alcohol | 5-8 seconds |
| o Safranin (1% aqueous) | 1 minute |

Specimens for the Gram stain procedure were derived from pure cultures incubated for 24 hours on Blood Agar isolation plates. "Calgiswabs" were used to transfer cellular material from a single colony to standard glass slides containing a drop of sterile water. The dispersed samples were fixed with gentle heat (40-45°C) for a minimum of 30 minutes before initiating the staining sequence. Cover slips were permanently affixed on each slide using "Permunt"⁶.

⁵New York Agricultural Experiment Station Technical Bulletin, 1927, page 128.

⁶Obtained from Fisher Scientific Company, Cat. No. So-P-15.

6.4 RAW DATA FROM LIVE SUBJECT TESTING

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MICROBIAL IDENTIFICATION

CODE NUMBER: D-2
SUBJECT NAME: Dice weight
SITE: 1 - Throat
SWAB: MOIST - DRY -
MEDIUM -
DATE: 8/10/71 TIME: 1415

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:
TAXO A - PRESENT ABSENT
TAXO P - PRESENT ABSENT
NUMBER OF APPARENT COLONY TYPES: 2
AROMA (IF PRESENT)

DESCRIPTION:
Red 30 - Enhance of white colony not
x hemol. white
OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS						HEMOL.
		CATAL- ASE	CYTO- OXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G	
1-a	47c <i>Streptococcus</i>	Yes	ND	white	2	1	3	2	1-3	
* 1-b	47c <i>Streptococcus</i>	Yes	ND	white	2	1	3	1	2	

* No isolated colonies available - May be mixed culture (8/11/91)

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER				PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	
1-a					Diphtheria group
1-b					Micrococcus

1. Very pleomorphic, short/long chain of high power, short fat forms, long, etc.
2. Mostly small, fairly uniform size

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- B. COLOR
- As Noted
- C. FORM
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

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MICROBIAL IDENTIFICATION

CODE NUMBER: D.W.
 SUBJECT NAME: Dave Wright
 SITE: 2 - Flares
 SWAB: MOIST - DRY - ✓
 MEDIUM - N13
 DATE: 8/10/71 TIME: 1415

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT ✓

TAXO P - PRESENT ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

DESCRIPTION: No 30 complete inhib.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.				COLONY CHARACTERISTICS			
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE		B	C	D	E
2-a	g/c yellow	POS.	ND	ND	white	2	1	3	1
2-b	g/c dispersed	POS.	ND	ND	white	2	1	3	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- As Noted
- C. FORM
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
2-a						Staph
2-b						Staph/Micrococci

① Uniform zone for some short chains, singles
 ② In all configurations 1, 2, 4, short chains, small chains

MICROBIAL IDENTIFICATION

CODE NUMBER: D.W.
 SUBJECT NAME: Paul Wright
 SITE: 3-2 Skin (Genarm)
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/16/71 TIME: 1415

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT
 TAXO P - PRESENT ABSENT
 NUMBER OF APPARENT COLONY TYPES: 1
 AROMA (IF PRESENT)
 DESCRIPTION: NW 30 - T F T T
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Pen To Tell

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-ASE	FORM	EDGE	ELEVATION	CHAR.	OPTICAL	HEMOL.
3-2	Stc Smth V	Pos	ND	white	2	1	3	1	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamdospores
- B. COLOR
- C. FORM
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
3-2							Staph

- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: G-F.
 SUBJECT NAME: Ge-don Fogel
 SITE: 3 - Throat
 SWAB: MOIST - ✓ DRY - ✓
 MEDIUM - ✓
 DATE: 8/10/71 TIME: 1330

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ✓ ABSENT ✓
 TAXO P - PRESENT ✓ ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 3
 AROMA (IF PRESENT)
 DESCRIPTION: Neo 30 B.T. h.b. 7 of hemol. colony.
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F
	GRAM STAIN REACTION & CELLULAR MORPHOLOGY			FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.
1-a	stc swab, very	neg	ND	white	2	1	3	2
1-b	stc swab, very	neg	ND	white	2	1	3	2
1-c	stc swab, very	neg	ND	white	2	1	4	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Commas
 - Spirals
 - Branched Rods
 - Mycelium
 - Chlamydospores
- B. COLOR
- As Noted
- C. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- D. EDGE
- Entire
 - Undulate
 - Lobate
 - Erose
 - Filamentous
 - Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AH 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
1-a						<u>Capnocytophaga</u>
1-b						<u>Staphylococcus</u>
1-c						<u>Staphylococcus</u>

- many single, double form, diplo config.
- Small clumps, over. Disorganized (gram -ve), irreg. size
- Strongly g+, irreg. size, short chains, generally small in size.

MICROBIAL IDENTIFICATION

CODE NUMBER: G. #1
 SUBJECT NAME: Golden Fagol
 SITE: 2-Nares
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/17/71 TIME: 1230

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT ✓
 TAXO P - PRESENT ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT)

DESCRIPTION:
New 3d - complete ink
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.				COLONY CHARACTERISTICS			
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-ORIDASE		B	C	D	E
2-a	97c <u>rod</u>	<u>pos</u>	<u>ND</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>3</u>	<u>1</u>
2-b	<u>mixed culture</u>	<u>pos</u>	<u>ND</u>	<u>ND</u>	<u>white</u>	<u>1</u>	<u>1</u>	<u>3</u>	<u>2</u>

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
 1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydozoospores
- B. COLOR
 As Noted
- C. FORM
 1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
 1. Entire
 2. Undulate
 3. Lobate
 4. Erose
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2-a							<u>Staph/micrococci</u>
2-b							<u>colony lactarium</u>
-2							<u>2</u>

- ① Strongly 97c, larger, simpler cocci.
 ② a) large, club-shaped, strongly 97c rod, strong staining
 b) slender 97c rod, some a wavy-like body one end of cell, variable length, some d. 1.5-2.0 µm.

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MICROBIAL IDENTIFICATION

CODE NUMBER: G. F.
 SUBJECT NAME: Gordon Fogel
 SITE: 3-Skin (foam)
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/12/71 TIME: 1330

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT
 TAXO P - PRESENT ABSENT
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT)
 DESCRIPTION: Wet - T F T T
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Few Tails

Too

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.
3-a	g+c - clumps	Pos	ND	white	2	1	3	1
3-b	g+c - multiple	Neg	ND	gray	1	1	3	1

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

- Cocci
- Diplococci
- Rods
- Comas
- Spirals
- Branched Rods
- Mycelium
- Chlamydospores

C. FORM

- Punctiform
- Circular
- Filamentous
- Irregular
- Rhizoid
- Spindle

D. EDGE

- Entire
- Undulate
- Lobate
- Erode
- Filamentous
- Curled

B. COLOR

As Noted

E. ELEVATION

- Effuse
- Flat
- Raised
- Convex
- Umbonate

F. OPTICAL CHARACTERISTICS

- Opaque
- Translucent
- Opalescent
- Irridescent

G. HEMOLYSIS

- Positive
- Negative
- Alpha
- Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
3-a						Staph.
3-b						Staph. aureus

- Strongly g+, large, imbricate, single, double, tails, etc.
- Strongly g+, irregular, some single, short chains

MICROBIAL IDENTIFICATION

CODE NUMBER: P.M.
 SUBJECT NAME: Peter Marchetta
 SITE: 1 - Throat
 SWAB: MOIST - ✓ DRY - ✓
 MEDIUM - ✓
 DATE: 8/17/71 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ✓ ABSENT ✓

TAXO P - PRESENT ✓ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT)

~~DESCRIPTION~~
new 30 - all head. colonies not inhibited

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G
1-a	g diplococcus	Neg	ND	1-2	1	3	2	1-3	
1-b	g c & g c & g c & g c	Neg	ND	2	1	3	1	2	
1-c	g c as above mixed smear	Neg	ND	6	6	5	1	2	

* Colonies not isolated - may be mixed sample / smear

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Commas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER				PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	
1-a					diplococcus / strap
1-b					strap / amonous
1-c					strap

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

- Small, coccoid shape, envelope present
- Some small clusters, slightly over submerged, possible size, some large, strongly g c about - mixed culture
- Evidence of out - disorganization, short chains of bacilli, some bunches, small, ovalish cocci

MICROBIAL IDENTIFICATION

CODE NUMBER: P-11
 SUBJECT NAME: Peter Marchetta
 SITE: 2 - Nares
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/12/71 TIME: 1340

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT ✓
 TAXO P - PRESENT ABSENT
 NUMBER OF APPARENT COLONY TYPES: 1- (w/ 2 color forms)
 AROMA (IF PRESENT)
 DESCRIPTION: sec 30 - complete inhib.
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G
2-a	g ⁺ c large, uniform	POS	ND	yellow	2	1	3	1	2
2-b	g ⁺ c large, uniform	POS	ND	white	2	1	3	1	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
- Cocci
 - Diplococci
 - Rods
 - Commas
 - Spirals
 - Branched Rods
 - Mycelium
 - Chlamydospores
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- Entire
 - Undulate
 - Lobate
 - Erose
 - Filamentous
 - Curled

- B. COLOR As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2-a							staph
2-b							staph

① mostly singles & chains, some short chains
 ③ As above

MICROBIAL IDENTIFICATION

CODE NUMBER: P-M
 SUBJECT NAME: Peter Marchetta
 SITE: 3-Skin (forearm)
 SWAB: MOIST - DRY -
 MEDIUM - NB
 DATE: 8/17/71 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT

TAXO P - PRESENT ABSENT

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

DESCRIPTION:

NEW-30 - T F T T

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

few To Tell
Tod
2 colonies

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS					F	G
		CATAL-ASE	CYTO-OXIDASE	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.		
3-a	g ⁺ rods	Pos	ND	gray	4	4	3	1	1	2
3-b	g ⁺ c large uniform	Pos	ND	yellow	2	1	3	1	1	2
3-c	g ⁺ c large clumps	Pos	ND	white	2	1	3	1	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

- Cocci
- Diplococci
- Rods
- Commas
- Spirals
- Branched Rods
- Mycelium
- Chlamydospores

C. FORM

- Punctiform
- Circular
- Filamentous
- Irregular
- Rhizoid
- Spindle

D. EDGE

- Entire
- Undulate
- Lobate
- Erode
- Filamentous
- Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
3-a						<i>coagulase</i>
3-b						<i>staph</i>
3-c						<i>staph</i>

G. HEMOLYSIS

- Positive
- Negative
- Alpha
- Beta

F. OPTICAL CHARACTERISTICS

- Opaque
- Translucent
- Opalescent
- Iridescent

E. ELEVATION

- Effuse
- Flat
- Raised
- Convex
- Umbonate

- large strongly g⁺ rods, much incidence of palisading
- heavy g⁺ c, uniform, singly & small clumps
- large clumps, strongly g⁺, almost all in clumps large cocci

MICROBIAL IDENTIFICATION

CODE NUMBER: JC
 SUBJECT NAME: John Cary
 SITE: I - Throat
 SWAB: MOIST - DRY -
 MEDIUM -
 DATE: 8/16/71 TIME: 1400

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT
 TAXO P - PRESENT ABSENT
 NUMBER OF APPARENT COLONY TYPES: 3
 AROMA (IF PRESENT)
 OBSERVATION:
Neo 30 - No inhibition of a hemol. colonies
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.			COLONY CHARACTERISTICS				
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F
1-a	g ⁺ small coccoid	Neg	Neg	Neg	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.
1-b	g ⁺ coccoid, chains	Neg	Neg	Neg	white	2	1	3	2
1-c	g ⁺ coccoid, chains	Neg	Neg	Neg	grey	2	1	3	1
						4	4	5	1

PROBABLY Staphylococcus aureus (after observation)

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Comas
 - Spirals
 - Branched Rods
 - Mycelium
 - Chlamydospores
- B. COLOR
- C. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- D. EDGE
- Entire
 - Undulate
 - Lobate
 - Erose
 - Filamentous
 - Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/H 300 mcg	
1-a						Diplo / strip
1-b						strip / amoxicillin
1-c						strip

- Some ~~short~~ chains, very small cocci, some diplo form: difficult to distinguish
- Strongly gram neg. small cocci, neg in 5% c.
- Large, strongly g⁺ cocci, some variability in size

- E. ELEVATION
- Effuse
 - Flat
 - Raised
 - Convex
 - Umbonate
- F. OPTICAL CHARACTERISTICS
- Opaque
 - Translucent
 - Opalescent
 - Irridescent
- G. HEMOLYSIS
- Positive
 - Negative
 - Alpha
 - Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: JC
 SUBJECT NAME: John Cary
 SITE: 2-Nares
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/16/71 TIME: 1400

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT ✓

TAXO P - PRESENT ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

DESCRIPTION:

New 30 - complete white.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

B-hemolytic colony inhibition

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A		BIOCHEM.		COLONY CHARACTERISTICS				
					B	C	D	E	F
	GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE	FORM	COLOR	EDGE	ELEVATION	CHAR.	HEMOL.
2-a	g+c streptococcus	Pos	ND	white	2	1	3	1	1-4
2-b	g+c streptococcus	Pos	ND	white	2	1	3	1	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- As Noted
- C. FORM
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
2-a						staph/micrococ
2-b						

- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

2 large areas, staphylococcus

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MICROBIAL IDENTIFICATION

CODE NUMBER: J.C.
 SUBJECT NAME: John Carey
 SITE: 3 - Skin (feram)
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/10/71 TIME: 1400

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT

TAXO P - PRESENT ABSENT

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT)

DESCRIPTION:

New 3d - TET

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Ten To Tell

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F
3-a	etc. clumps	Pos	ND	white	2	1	3	1

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Comas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamydozoetes

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erode
5. Filamentous
6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
3-a						Staph

① Suspended organism. strongly g+

①

MICROBIAL IDENTIFICATION

CODE NUMBER: TC
 SUBJECT NAME: Japanese Cherry
 SITE: 1 - Throat
 SWAB: MOIST - — DRY - ✓
 MEDIUM - —
 DATE: 8/14/71 TIME: 1345

+ diplococci in chains, some quite large;
 variable staining (chain); not like penicillium

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT — ABSENT ✓

TAXO P - PRESENT — ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT) N/A

DESCRIPTION: Non-invasive. Inhibition of white colonies but not colorless/pigmented (like penicillium)
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	BIOCHEM.				COLONY CHARACTERISTICS			
	A	CATAL-ASE	CYTO-OXIDASE	B	C	D	E	G
1a	Gram stain reaction & cellular morphology	Neg	ND	white	2	1	3	2
1b	Gram stain reaction & cellular morphology	Neg	ND	white	2	1	3	1
1c	Gram stain reaction & cellular morphology	Neg	ND	gray	2	1	3	1

DESCRIPTIVE KEYS:

1. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Commas
 - Spirals
 - Branched Rods
 - Mycelium
 - Chlamydozoetes
2. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
3. EDGE
- Entire
 - Undulate
 - Lobate
 - Erose
 - Filamentous
 - Curled

B. COLOR

As Noted

E. ELEVATION

- Effuse
- Flat
- Raised
- Convex
- Umbonate

F. OPTICAL CHARACTERISTICS

- Opaque
- Translucent
- Opalescent
- Iridescent

G. HEMOLYSIS

- Positive
- Negative
- Alpha
- Beta

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SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM	P	N	G	Te	
10 mcg	10 u	30 mcg	2 mg	F/M	300 mcg	30 mcg

① Over decolorized, but definitely 9+ - no chains per chains, most organisms or bacilli - make some look like short rods
 ② Non-uniform formation - many are. color showing 9+ color - probably over decolorized i.e. 9+ not 9+ as many are demonstrating SC = subculture

MICROBIAL IDENTIFICATION

CODE NUMBER: TE - 2
 SUBJECT NAME: Theresa Cherry
 SITE: 2 - Nares
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/14/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT ✓
 TAXO P - PRESENT ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT)

DESCRIPTION: New 30% complete in L. bitum. but disc.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F
2-a	GC 4.5% <u>2.5% 1.5% 1.0% 0.5%</u>	ND	ND	2	1	3	1	1-4
2-b	GC 4.5% <u>2.5% 1.5% 1.0% 0.5%</u>	ND	ND	1	1	3	1	2

DESCRIPTIVE KEYS:

- CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
1. Entire
 2. Undulate
 3. Lobate
 4. Erose
 5. Filamentous
 6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg
2-a						Staph
2-b						crystalline

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

③ disk changed, stumpy g⁺, great variability in size

MICROBIAL IDENTIFICATION

CODE NUMBER: 7C (3)
 SUBJECT NAME: Theresa Cherry
 SITE: 3 Skin (forearm)
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/11/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT

TAXO P - PRESENT ABSENT

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT) W/A

DESCRIPTION:

Wet 30 - Too few colonies to tell

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.			COLONY CHARACTERISTICS					G
		CATAL-ASE	CYT-OXIDASE	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.	HEMOL.	
3a ①	9+ r, 9- r, ③ Pcs			white	2	1	3	1	2	

DESCRIPTIVE KEYS:

CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Comas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamydospores

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	C 2 mcg	F/H 300 mcg	Te 30 mcg
3a 1						
3a 2						

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

57

① Mixed culture? - large, strongly 9+ rod in variable staining and club shaped appearance.
 ② Slender, 9- rod showing possible pleomorphism - possibly indicates difference in rate of decolorization.

colonies
 pink to red

1-a - very sparse growth at 24 hrs. - only colonies visible - appears to be pure culture at 24 hrs. - No hemolysis
 48 hrs - No change

MICROBIAL IDENTIFICATION

CODE NUMBER: TC - 2 ①

SUBJECT NAME: Theresa Cherry

SITE: Subculture 1-a 1-c
 1-b 3-a

SWAB: MOIST - DRY

MEDIUM - DRY

DATE: 8/10/71 TIME: 1600

PRIMARY CULTURE INFORMATION - 1-c culture

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT

TAXO P - PRESENT ABSENT

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT) None

DESCRIPTION: None

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.) None

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	FORM	COLON	EDGE	ELEVATION	CHAR.
1-a ①	9+c single +	Pos ±	ND	white	2	1	3	2
1-b ②	9+c single +	Neg	ND	white	2	1	3	1
1-b ③	9+c single +	Neg	ND	white	2	1	3	1
1-c ④	9+c single +	Neg	ND	white	2	1	3	1
3-a ⑤	9+c single +	Pos ±	ND	white	2	1	3	1
3-a ⑥	9+c single +	Neg	ND	white	2	1	3	1

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
1. Entire
 2. Undulate
 3. Lobate
 4. Erose
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	
1-a						<u>Micrococcus</u>
1-b						<u>Strep</u>
1-b ②						<u>Strep</u>
1-c						<u>Strep</u>

- ① Some short chains, strongly 9+c single, mostly in doublets
- ② large, uniform size, some short chains
- ③ Strongly Gram +, easily confused size
- ④ large, strong by 8 um + some short chains
- ⑤ Some club shape, some slight polar body
- ⑥ Contain strongly 9+c short fat rods

- E. ELEVATION F. OPTICAL CHARACTERISTICS G. HEMOLYSIS
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
1. Positive
 2. Negative
 3. Alpha
 4. Beta

1-b - Heavy growth, appears to be a gray/blue pigment present at area of heart in center. large, milky, shiny colonies and small, milky colonies difficult to say if 2 types or 1 at 24 hrs. No hemolysis

48 hrs - Probably pure culture - degree of counting makes difference in colonies appearing in order of 50%.

MICROBIAL IDENTIFICATION

CODE NUMBER: 11
 SUBJECT NAME: garc laxsack
 SITE: 1 - Throat
 SWAB: MOIST - DRY - ✓
 MEDIUM -
 DATE: 8/14/71 TIME: 1410

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT ✓
 TAXO P - PRESENT ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT)
 DESCRIPTION: white pipe-hyphal
 New 35 inhibition 7
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
white colonies few in number - not related
for every area

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.		COLONY CHARACTERISTICS				
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE	B	C	D	E
1-a	Q 9 red in blood	rod	ND	ND	2	1	3	1-3
1-b	Q 9 red in blood	rod	ND	ND	2	1	4	2
48 hr	Q 9 red in blood	rod	ND	ND	4	3	5	2
1-c	Q 9 red in blood	rod	ND	ND	4	3	5	2

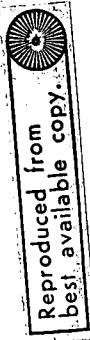
* May be mixed culture - no isolated colonies available

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
1						last box
2						
3						
4						
5						

- Very difficult to distinguish between rod & diplococci - Strongly 9 + some suggestion of pleomorphism and small, club-shaped appearance, possibly variable staining pattern
- Great variability in size - some oval - some round - some pointed culture
- uniform size - no evidence of mixed culture

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Comas
 - Spirals
 - Branches Rods
 - Mycelium
 - Chlamydospores
- B. COLOR
- As Noted
- C. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- D. EDGE
- Entire
 - Undulate
 - Lobate
 - Krose
 - Filamentous
 - Curled
- E. ELEVATION
- Effuse
 - Flat
 - Raised
 - Convex
 - Umbonate
- F. OPTICAL CHARACTERISTICS
- Opaque
 - Translucent
 - Opalescent
 - Irridescent
- G. HEMOLYSIS
- Positive
 - Negative
 - Alpha
 - Beta



MICROBIAL IDENTIFICATION

CODE NUMBER: 56
 SUBJECT NAME: Jane Kayack
 SITE: 2 - Nares
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/11/71 TIME: 1410

(2)

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT ✓
 TAXO P - PRESENT ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT) N/A
 DESCRIPTION: New 30 - Complete inhibition
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.	HEMOL.
2-a	G+C <u>no. 111</u> <u>chapters</u>	<u>pos</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>3</u>	<u>1</u>	<u>1-4</u>
2-b	G+C <u>no. 111</u> <u>chapters</u> <u>and</u> <u>samples</u>	<u>pos</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>3</u>	<u>1</u>	<u>2</u>

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Comas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamydospores

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER						PRESUMPTIVE ID
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2-a							<u>Staph</u>
2-b							<u>Staph/micrococcus</u>

① uniform size
 ② Chrysem size

MICROBIAL IDENTIFICATION

CODE NUMBER: 36
 SUBJECT NAME: Jane Laycock
 SITE: 3 - Skin (Forearm)
 SWAB: MOIST - ✓ DRY -
 MEDIUM - MB
 DATE: 8/11/71 TIME: 1410

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT
 TAXO P - PRESENT ABSENT
 NUMBER OF APPARENT COLONY TYPES:
 AROMA (IF PRESENT) N/A
 OBSERVATION:
new 30 - N/A 24 hrs.
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
No apparent growth in 24 hrs.

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G
1									
2									
3									
4									
5									
6									
7									
8									

DESCRIPTIVE KEYS:

- CELLULAR MORPHOLOGY
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- C. FORM
1. Entire
 2. Undulate
 3. Lobate
 4. Erose
 5. Filamentous
 6. Curled
- D. EDGE
- As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
1							
2							
3							
4							
5							
6							
7							
8							

- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

24 hrs. 1-a Pure culture at 24 hrs. good growth to large circular colonies showing strong α hemol.
48 hrs. NO change

MICROBIAL IDENTIFICATION

CODE NUMBER: J. L. 7-2-2 Ranbham
 SUBJECT NAME: Jane Kaye
 SITE: Subculture 4-a
 SWAB: MOIST - 2-b DRY - 2-b
 MEDIUM - 2-b
 DATE: 8/16/71 TIME: 1600

④

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT

TAXO P - PRESENT ABSENT

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT) None

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G
1-a	gtc diplococci	neg	ND	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.	HEMOL.
4-b, ②	gtc diplococci	neg	ND	Gray	2	1	3	1	2-3
1-b, ③	gtc R. Var. staining	Pos	ND	Gray	2	1	3	1	2-3

* No isolated colonies available - probably mixed smear on gram stain

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	
1-a						diplococci
1-b-1						diplococci
1-b-2						organism

- ① Some short chains, partially dehydrated, some pleomorphic forms
- ② Some short chains of diplo form
- ③ Pleomorphic forms - club shape, some long slender, others short fat, evidence of pathogenicity

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- As Noted
- C. FORM
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

DESCRIPTIVE KEYS:

- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

1-b Mixed culture @ 24 hrs. ① large circular, opaque colonies w strong α hemol. mixed w other ② punctiform colonies in yeast abundance no evidence of hemol.

48 hrs - NO change

MICROBIAL IDENTIFICATION

CODE NUMBER: FR
 SUBJECT NAME: Fred Rudek
 SITE: 1 Throat
 SWAB: MOIST - DRY - ✓
 MEDIUM -
 DATE: 8/14/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (1924 HOURS)

TAXO DISC REACTION: no effect

TAXO A - PRESENT ✓ ABSENT

TAXO P - PRESENT ✓ ABSENT

NUMBER OF APPARENT COLONY TYPES: 3-4

AROMA (IF PRESENT)

DESCRIPTION:

Nice 30 - inhib. vs. a & c

Type d in very small numbers - Too few to tell.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Some apparent colony types for in specimen and not isolated for sampling

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION
1-a	<u>Opt c moty samples</u>	<u>Neg</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>3</u>
1-b	<u>Opt c chains</u>	<u>Neg</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>3</u>
1-c	<u>Opt c samples</u>	<u>Neg</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>5</u>
1-d	<u>Opt c chains</u>	<u>Pos</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>4</u>

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Comas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamydozoetes

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg
1-a						
1-b						
1-c						
1-d						

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

63

① over decubating zone short chains, pink uniform size
 ② Very irregular staining granular size. many smaller, difficult to stain to interpret
 ③ Some smears, no discernible clumps
 SC = Subculture

MICROBIAL IDENTIFICATION

CODE NUMBER: FR

SUBJECT NAME: FR Red Rudek

SITE: 2 - Nares

SWAB: MOIST - ✓ DRY - ✓

MEDIUM - NB

DATE: 8/14/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ✓ ABSENT ✓TAXO P - PRESENT ✓ ABSENT ✓NUMBER OF APPARENT COLONY TYPES: 2AROMA (IF PRESENT) N/A~~DESCRIPTION~~Neo 30 - complete in lip

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.			COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	FORM	B	C	D	E
2-a	Q ₁ Van C <u>Staph</u>	Pos	ND	white	2	1	3	1
2-b	Q ₁ <u>g⁺c clusters</u>	Pos	ND	white	2	1	3	1

DESCRIPTIVE KEYS:

CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Comas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2-a							<u>Staph</u>
2-b							<u>Staph/micrococci</u>

① occur mostly in packets, few singles

② Uniform size

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: FR
 SUBJECT NAME: Fred Rudek
 SITE: 3 Skin (fascia)
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/4/71 TIME: 1330

(3)

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT
 TAXO P - PRESENT ABSENT
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT) N/A
 DESCRIPTION: New 30-7 FTT
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Too few colonies to tell

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.			COLONY CHARACTERISTICS				HEMOL.
		CATAL-ASE	CYTO-OXIDASE	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.	
3-a	<i>Qgtc - lactose</i>	<i>Pos</i>	<i>ND</i>	<i>white</i>	<i>2</i>	<i>1</i>	<i>3</i>	<i>1</i>	<i>2</i>
3-b	<i>Qgtc - lactose, 25</i>	<i>Pos</i>	<i>ND</i>	<i>yellow</i>	<i>2</i>	<i>1</i>	<i>3</i>	<i>1</i>	<i>2</i>

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
 1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydozoetes
- B. COLOR
 As Noted
- C. FORM
 1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
 1. Entire
 2. Undulate
 3. Lobate
 4. Erose
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	F 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
3-a						<i>Staph</i>
3-b						<i>Staph/micrococci</i>

- ① Small size (is suspect to 2 b. line)
 ② Great variability in size & shape - some gram variability

CODE NUMBER: 1 R - 2
SUBJECT NAME: Fred Rudels
SITE: Subculture 1-b
SWAB: MOIST - DRY -
MEDIUM -
DATE: 8/16/71 TIME: 1600

PRIMARY CULTURE INFORMATION *K. culture*

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION: _____

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: _____

AROMA (IF PRESENT) _____

DESCRIPTION: _____

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.) _____

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS					
		CATAL- ASE	CYTO- OXIDASE	B	C	D	E	F	G
1-b ①	g+c ²⁻¹⁵⁻⁵ 2-15-5 ²⁻¹⁵⁻⁵	Neg	ND	Form	Color	Edge	Elevation	Optical Char.	Hemol.
1-b 2 ②	g-r pleomorphic	Neg	ND	lobes	2	1	3	2	2-3
1-b 3 ③	g+c single and g+c tandem	Pos	ND	white	4	1	3	3	2
1-c ④	g+c singles, double	Neg	ND	white	2	1	3	1	2

A. CELLULAR MORPHOLOGY	C. FORM	D. EDGE
1. Cocci	1. Punctiform	1. Entire
2. Diplococci	2. Circular	2. Undulate
3. Rods	3. Filamentous	3. Lobate
4. Commas	4. Irregular	4. Erose
5. Spirals	5. Rhizoid	5. Filamentous
6. Branched Rods	6. Spindle	6. Curled
7. Mycelium		
8. Chlamydospores		

As Noted

single 9th predominates

E. ELEVATION		F. OPTICAL CHARACTERISTICS		G. HEMOLYSIS	
1.	Effuse	1.	Opaque	1.	Positive
2.	Flat	2.	Translucent	2.	Negative
3.	Raised	3.	Opalescent	3.	Alpha
4.	Convex	4.	Iridescent	4.	Beta

1.c. Difficult plate to read at 24 hrs. large, ivory and smooth
24hrs. round ivory columns to small, shiny, ivory, white
columns - inhibited version of large due to crowding
or 2 distinct variants. 2

48 hrs. - Carbonate colonies quite smooth on periphery of ~~disk~~ 5 mm. - crowded portion has smooth ^{irregular} surface

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- ① somewhat gem variable, bubble form predominates,
- ② some forms long, slender 9" or others fairly short & fat. also evidence of some 4" & about in small cone. - cones were not separated.
- ③ Uniform c. some short chain

MICROBIAL IDENTIFICATION

CODE NUMBER: MK
 SUBJECT NAME: Martin Koesterer
 SITE: 2 - Hawaii
 SWAB: MOIST - ✓ DRY - ✓
 MEDIUM: MA
 DATE: 8/9/71 TIME: 1340

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ✓ ABSENT ✓
 TAXO P - PRESENT ✓ ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 3-4
 AROMA (IF PRESENT)
~~DESCRIPTION~~
 Miconycin 30 - Apparent inhibition of white, opaque colonies in 3 exceptions (exceptions not sampled for testing)
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
 poor dispersion of colonies difficult to find isolated colonies

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.		COLONY CHARACTERISTICS				
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE	B	C	D	E
1a	94c ^{unpigmented, small, 4-5 diam}	ND	ND	ND	white	2	1	3
1b	94c ^{dytic, small}	Uy	ND	ND	white	2	1	3
1c	94c ^{rod pleomorphic}	Uy	ND	ND	ivory	4	4	5

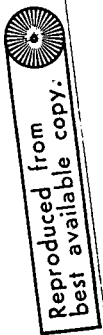
DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Commas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- As Noted
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	F 10 u	N 30 mcg	G 2 mcg	Te 30 mcg	
1-a						Slough / micrococci
1-b						Slough / diplococci
1-c						2

- E. ELEVATION F. OPTICAL CHARACTERISTICS G. HEMOLYSIS
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
1. Positive
 2. Negative
 3. Alpha
 4. Beta

1. very sparse slide, not over embedded to organisms. there are
 2. exhibit practically every type of arrangement possible
 3. Shows tendency to decolorize easily & mostly singly (in dyed configuration) some short chains
- ③ few organisms on slide - some appear as granular - spaces between stained areas on w/d.
 quite visible as to size



MICROBIAL IDENTIFICATION

CODE NUMBER: MK
SUBJECT NAME: Martin Kaestner
SITE: 2 - Nares
SWAB: MOIST - ✓ DRY -
MEDIUM - MB
DATE: 7/20/71 TIME: 1340

PRIMARY CULTURE INFORMATION
RESULTS ON BLOOD AGAR (18-24 HOURS)
TAXO DISC REACTION:
TAXO A - PRESENT ABSENT ✓
TAXO P - PRESENT ABSENT ✓
NUMBER OF APPARENT COLONY TYPES: 3 (2 type not available for testing at 24 hrs.)
AROMA (IF PRESENT) dec
DESCRIPTION: Neomycin 30: complete inhibition surrounding
OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.) dec

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G	H
2a	Gram +ve cocci in pairs	Pos	ND	white	4	1	3	1	1	1
2b	Gram +ve cocci in chains	Pos	ND	white	2	1	3	1	1	1
2c	Gram +ve cocci in chains	Pos	ND	white	1	1	3	1	1	1
2d	Gram -ve rods	ND	ND	white	2	1	3	1	1	1

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
 2. Diplococci
 3. Rods
 4. Comma
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- C. FORM
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled
- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2a							Staph
2-b							Staph
2-c							2
2-d							Corynebacterium

② Somewhat variable in size - much evidence of tendency to desiccate.

④ Either overdehydrated or strongly gram variable. Rods show much evidence of septal staining in what appears to be sphere-like chains. Need to look closely to determine all wall of rod.

MICROBIAL IDENTIFICATION

CODE NUMBER: MS
 SUBJECT NAME: Martin Koesterer
 SITE: 3 - Skin (Forearm)
 SWAB: MOIST - 6 DRY -
 MEDIUM - MB
 DATE: 8/9/71 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT

TAXO P - PRESENT ABSENT

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) NA

DESCRIPTION:

Penicillin 30 - Two sec! - (no colony growth)

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Two sec colonies to determine reaction positively

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F
3a	<u>Opt large cocci</u>	<u>POS</u>	<u>ND</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>3</u>	<u>1</u>
3b	<u>much of them no growth if that is organism</u>			<u>white</u>	<u>4</u>	<u>4</u>	<u>3</u>	<u>2-3</u>

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Commae
5. Spirilla
6. Branched Rods
7. Mycelium
8. Chlamydozoetes

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

B. COLOR

As Noted

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
3a							<u>staph</u>
3b							<u>None</u>

* 48 hrs - some colonies show beta hemolytic halo around; others of same appeared shorter color is not.
 Very large spheres, mostly chains, but some chains. Some are 1's, 2's, and 4's, uniform size

MICROBIAL IDENTIFICATION

CODE NUMBER: J.M.
 SUBJECT NAME: Joby Mangialardi
 SITE: 1 - Throat
 SWAB: MOIST - ✓ DRY - ✓
 MEDIUM - NA
 DATE: 8/9/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-22 HOURS)

TAXO DISC REACTION:
 TAXO A - PRESENT ✓ ABSENT ✓
 TAXO P - PRESENT ✓ ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT) W/A

DESCRIPTION: inhibition of white colonies, no apparent effect on agar, colorless ones.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.				COLONY CHARACTERISTICS			
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.
1a	White clumps, 1st	Neg	ND	White	2	1	3	2	1-2
1b	White clumps, 2nd	Neg	ND	White	2	1	3	2	1-3

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydozooids
- B. COLOR
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
1-a							Strep penicillino
1-b							Strep pyogenes

- ① Evidence of variable size & shape; also tendency to show some evidence of decolorization
- ② Some short chains, organisms generally small and oval shape

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MICROBIAL IDENTIFICATION

CODE NUMBER: 101
 SUBJECT NAME: John Mangiaardi
 SITE: 2 - Wares
 SWAB: MOIST - ✓ DRY -
 MEDIUM - 1/B
 DATE: 8/9/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT ✓

TAXO P - PRESENT ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

DESCRIPTION:

Neomycin 30 - complete inhibition around 1/30 disc

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.			COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	FORM	B	C	D	E	F
2a	Dgtc warty clumps	Pos	ND	white	2	1	3	1	2
2b	Dgt Red p. conophial	Weg	ND	white	1	1	2	2	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Comas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamydospores

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE ID
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2a							Staph
2b							conophial

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

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- ① Strong tendency to decolorize, much variability in size, few samples
- ② Strongly 9⁺, very variable in shape: small ovoid rods to large (3-4 x 1.4) club-shaped rods. Additional staining: S. pyogenes, S. aureus on same colony of 48 hr. incub. showed large, club-shaped rods (detected to be spores by negative 9⁺ the size in relation to rods in which it existed) many, club-shaped rods visible.

MICROBIAL IDENTIFICATION

CODE NUMBER: J. M.
 SUBJECT NAME: John Mangialardi
 SITE: 3-Skin Cream
 SWAB: MOIST - ✓ DRY - —
 MEDIUM - NB
 DATE: 8/9/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT — ABSENT —

TAXO P - PRESENT — ABSENT —

NUMBER OF APPARENT COLONY TYPES: —

AROMA (IF PRESENT) N/A

DESCRIPTION: None in 30 - 70 sec to tell

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Too few to tell
may be apparent
in 10 min

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-DIASE	B	C	D	E	F	G	H
3a	<u>1/2 in diam on 10% Te</u>	<u>neg</u>	<u>4D</u>	<u>4</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>2</u>	<u>2</u>	<u>2</u>

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
 2. Diplococci
 3. Rods
 4. Commae
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- As Noted
- C. FORM
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erose
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
3a							<u>None</u>

- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: J.S.
 SUBJECT NAME: John Scheelkopf
 SITE: 1 - Throat
 SWAB: MOIST - ✓ DRY - ✓
 MEDIUM: N/A
 DATE: 8/9/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ✓ ABSENT ✓

TAXO P - PRESENT ✓ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT) N/A

DESCRIPTION:

*Neomycin 30 - inhibition of white & ivory
opaque types not of the same*

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYT-OXIDASE	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.	HEMOL.
1a	<i>Gram + cocci in pairs, some chains</i>	ND	ND	<i>white</i>	<i>1-2</i>	<i>4</i>	<i>3</i>	<i>2</i>	<i>1-3</i>
1b	<i>Gram + cocci in pairs, some chains</i>	ND	ND	<i>white</i>	<i>2</i>	<i>1</i>	<i>3</i>	<i>1</i>	<i>2</i>
1c	<i>Gram + cocci in pairs, some chains</i>	ND	ND	<i>white</i>	<i>2</i>	<i>1</i>	<i>4</i>	<i>1</i>	<i>2</i>

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Commas
 - Spirals
 - Branching Rods
 - Mycelium
 - Chlamydospores
- B. COLOR
- As Noted
- C. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- D. EDGE
- Entire
 - Undulate
 - Lobate
 - Erose
 - Filamentous
 - Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
1-a						<i>Strep/diplococci</i>
1-b						<i>Strep/diplococci</i>
1-c						<i>Strep/diplococci</i>

DESCRIPTIVE KEYS:

- E. ELEVATION
- Effuse
 - Flat
 - Raised
 - Convex
 - Umbonate
- F. OPTICAL CHARACTERISTICS
- Opaque
 - Translucent
 - Opalescent
 - Irridescent
- G. HEMOLYSIS
- Positive
 - Negative
 - Alpha
 - Beta

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① cocci in the chains are smaller than those appearing in the single cc's, singles which appear large may be diplococci
 ② large cocci in all arrangements
 ③ also some long chains (10-15)

MICROBIAL IDENTIFICATION

CODE NUMBER: J.S.
 SUBJECT NAME: John Schalkopf
 SITE: 2-Nares
 SWAB: MOIST - ✓ DRY -
 MEDIUM: MB
 DATE: 8/9/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-25 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT
 TAXO P - PRESENT ABSENT

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT) NA

DESCRIPTION:

Penicillin 30 - complete inhibition of all growth except a small colony (micro)
Overall description of culture: (color, etc.)
Entire plate looks like monoculture w/ oblique light & small vegetation only. However, upon
chilled light - there are some flares in places
(hemolysis) & others up, some small pin
DESCRIPTION KEYS: whistles & lines

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.				COLONY CHARACTERISTICS			
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE		B	C	D	E
2a	g+c mostly chains	Pos				white	2	1-4	1
2b	g+c on alone	Pos				white	2	1	3
2c	g+c on alone	Neg				colorless	1-4	1	3

- DESCRIPTIVE KEYS: whistles & lines
- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci 1. Punctiform 1. Entire
 2. Diplococci 2. Circular 2. Undulate
 3. Rods 3. Filamentous 3. Lobate
 4. Comas 4. Irregular 4. Erose
 5. Spirals 5. Rhizoid 5. Filamentous
 6. Branched Rods 6. Spindle 6. Curled
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	C 2 mg	F/M 300 mcg	Te 30 mcg	
2-a							staph
2-b							staph/micrococci
2-c							staph/aerococcus

- E. ELEVATION F. OPTICAL CHARACTERISTICS G. HEMOLYSIS
1. Effuse 1. Opaque 1. Positive
 2. Flat 2. Translucent 2. Negative
 3. Raised 3. Opalescent 3. Alpha
 4. Convex 4. Irridescent 4. Beta
 5. Umbonate

MICROBIAL IDENTIFICATION

CODE NUMBER: J.S.
 SUBJECT NAME: John Schellkopf
 SITE: 3 - Skin (groin)
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/9/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ✓ ABSENT

TAXO P - PRESENT ✓ ABSENT

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

DESCRIPTION:

Nonhemolytic: 16 growth around disc

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

only one large white colony visible and
signifying very colorless colony at periphery
of medium swc. area.

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS				F OPTICAL CHAR.	G HEMOL.
		CATAL-ASE	CYTO-OXIDASE	B	C	FORM	COLOR	EDGE	ELEVATION		
3 a	<u>7c mostly 1/2-2's</u>	<u>POS</u>		<u>white</u>	<u>1</u>	<u>1</u>	<u>1</u>	<u>3</u>	<u>3</u>	<u>1</u>	<u>2</u>
3 b	<u>Ab circ dense 7 bact. Wey</u>			<u>white</u>	<u>4</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>2</u>	<u>2</u>

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamydospores

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

B. COLOR

As Noted

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
3-a							<u>Staph/Micrococcus</u>
3-b							<u>none</u>

MICROBIAL IDENTIFICATION

CODE NUMBER: EG
 SUBJECT NAME: Ed Glandfield
 SITE: 1 - throat
 SWAB: MOIST - ✓ DRY - ✓
 MEDIUM - ✓
 DATE: 8/3/71 TIME: 1345

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ✓ ABSENT ✓
 TAXO P - PRESENT ✓ ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 2-3 ?
 AROMA (IF PRESENT)
 DESCRIPTION:
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
white opaque colonies
colonies in 1 up to 1 mm - same organism?

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.		COLONY CHARACTERISTICS				
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE	B	C	D	E
1-a	<u>Gram Stain: cocci in pairs</u>	<u>Neg</u>	<u>N/D</u>	<u>N/D</u>	<u>white</u>	<u>2</u>	<u>1</u>	<u>4</u>
1-b	<u>Gram Stain: diplococci, long, single</u>	<u>Neg</u>	<u>N/D</u>	<u>N/D</u>	<u>color</u>	<u>4</u>	<u>1</u>	<u>3</u>
1-c	<u>Gram Stain: cocci in pairs, single</u>	<u>Neg</u>	<u>N/D</u>	<u>N/D</u>	<u>"</u>	<u>4</u>	<u>1</u>	<u>3</u>

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydozoetes
- B. COLOR
- As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	
1-a						<u>Staph</u>
1-b						<u>Staph/diplococci</u>
1-c						<u>Staph/aerobius</u>

- E. ELEVATION F. OPTICAL CHARACTERISTICS G. HEMOLYSIS
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
1. Opaque
2. Translucent
3. Opalescent
4. Irridescent
1. Positive
 2. Negative
 3. Alpha
 4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: EG
 SUBJECT NAME: Ed Glandfield
 SITE: 2 - Nose
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/3/71 TIME: 1345

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT ✓
 TAXO P - PRESENT ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 1
 AROMA (IF PRESENT) NA
 DESCRIPTION:
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
large white colonies

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.
2-a	<i>Grami</i> <i>long</i> <i>5</i>	POS	ND	white	2	1	4	1

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
- B. COLOR
- As Noted
- C. FORM
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
- D. EDGE
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2-a							<i>Staph aureus</i>

- E. ELEVATION
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
- F. OPTICAL CHARACTERISTICS
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
- G. HEMOLYSIS
1. Positive
 2. Negative
 3. Alpha
 4. Beta

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MICROBIAL IDENTIFICATION

CODE NUMBER: EG
 SUBJECT NAME: Ed. Glanfield
 SITE: 3 - Cream, Skin
 SWAB: MOIST - ✓ DRY - ✓
 MEDIUM - MB
 DATE: 8/3/71 TIME: 1345

PRIMARY CULTURE INFORMATION
 RESULTS ON BLOOD AGAR (18-24 HOURS)
 TAXO DISC REACTION:
 TAXO A - PRESENT ✓ ABSENT ✓
 TAXO P - PRESENT ✓ ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 2
 AROMA (IF PRESENT) NA
 DESCRIPTION:
 OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
Large white colonies
tiny colonies

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F
3-a	G. fac. 1995			white	2	1	3	1
3-b	G. fac. 1995			white	1	1	3	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamdospores
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
1. Entire
 2. Undulate
 3. Lobate
 4. Rose
 5. Filamentous
 6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
3-a						Staph
3-b						Staph

- E. ELEVATION F. OPTICAL CHARACTERISTICS G. HEVOLYSIS
1. Effuse
 2. Flat
 3. Raised
 4. Convex
 5. Umbonate
1. Opaque
 2. Translucent
 3. Opalescent
 4. Irridescent
1. Positive
 2. Negative
 3. Alpha
 4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: 56
 SUBJECT NAME: John Gaudin
 SITE: 1 - throat
 SWAB: MOIST - DRY - ✓
 MEDIUM -
 DATE: 8/3/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT ✓

TAXO P - PRESENT ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

dry appearance - some large colonies in yellow centers - rest white - small colonies in center

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G	H
1-a	G + some chains	slg	ND	slg	2	1	4	1	2	
1-b	G + some chains	slg	ND	slg	2	2	3	2	1-3	

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

1. Cocci
2. Diplococci
3. Rods
4. Comas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamydozoetes

C. FORM

1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE

1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

B. COLOR

As Noted

E. ELEVATION

1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS

1. Opaque
2. Translucent
3. Opalescent
4. Irridescent

G. HEMOLYSIS

1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
1-a							Slap/positive
1-b							Slap/positive

MICROBIAL IDENTIFICATION

CODE NUMBER: 56
 SUBJECT NAME: John Gearing
 SITE: 2-Nose
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/3/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT ✓

TAXO P - PRESENT ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Large white colonies some tiny, some thin colonies, colonies white to yellow and

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G	H
2-a	St cocci - chains	Pos	ND	Form: <i>short</i>	2	1	4	1	2	
2-b	" " clumps*	Neg	ND	Form: <i>long</i>	2	1	4	1	2	
2-c	" " clumps	Neg	ND	Form: <i>long</i>	1	1	3	2	2	

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY C. FORM D. EDGE
1. Cocci
 2. Diplococci
 3. Rods
 4. Comas
 5. Spirals
 6. Branched Rods
 7. Mycelium
 8. Chlamydospores
1. Punctiform
 2. Circular
 3. Filamentous
 4. Irregular
 5. Rhizoid
 6. Spindle
1. Entire
 2. Undulate
 3. Lobate
 4. Erode
 5. Filamentous
 6. Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
2-a							<i>Staph</i>
2-b							<i>Staph</i>
2-c							<i>Staph</i>

* Many over-distained (decolorized)

MICROBIAL IDENTIFICATION

CODE NUMBER: 56

SUBJECT NAME: John Greyling

SITE: 3 - Trueman, Skin

SWAB: MOIST - ✓ DRY -

MEDIUM - NB

DATE: 8/3/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT ✓

TAXO P - PRESENT ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

opaque colonies to yellow centers

Irres color

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A	BIOCHEM.				COLONY CHARACTERISTICS					
		GRAM STAIN REACTION & CELLULAR MORPHOLOGY	CATAL-ASE	CYTO-OXIDASE	FORM	B	C	D	E	F	G
3-a	G+ cocci chains	Neg	Neg	Neg	Irreg	Irreg	2	1	4	1	2
3-b	" " short chains	Neg	Neg	Neg	Irreg	Irreg	2	1	4	1	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Comas
 - Spirals
 - Branched Rods
 - Mycelium
 - Chlamydospores
- B. COLOR
- As Noted
- C. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- D. EDGE
- Entire
 - Undulate
 - Lobate
 - Erose
 - Filamentous
 - Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	
3-a							Sharp/Auricular
3-b							Sharp/Auricular

- E. ELEVATION
- Effuse
 - Flat
 - Raised
 - Convex
 - Umbonate
- F. OPTICAL CHARACTERISTICS
- Opaque
 - Translucent
 - Opalescent
 - Irridescent
- G. HEMOLYSIS
- Positive
 - Negative
 - Alpha
 - Beta



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MICROBIAL IDENTIFICATION

CODE NUMBER: 544
 SUBJECT NAME: Story Hunt
 SITE: 1-1 Hunt
 SWAB: MOIST - DRY - ✓
 MEDIUM -
 DATE: 8/2/71 TIME: 1315

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:
 TAXO A - PRESENT ABSENT ✓
 TAXO P - PRESENT ABSENT ✓
 NUMBER OF APPARENT COLONY TYPES: 3
 AROMA (IF PRESENT) Non-specific
 DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS				
		CATAL-ASE	CYTO-OXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.
1-a	G ⁺ Cocci clumps	Neg	ND	white	2	4	4	1
1-b	G ⁺ Diplococci, somewhat	Neg	ND	spores	4	1	4	2
1-c	G ⁺ Cocci short chains, mostly	ND	ND	dark	2	1	4	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY

- Cocci
- Diplococci
- Rods
- Commas
- Spirals
- Branching Rods
- Mycelium
- Chlamydozoetes

C. FORM

- Punctiform
- Circular
- Filamentous
- Irregular
- Rhizoid
- Spindle

D. EDGE

- Entire
- Undulate
- Lobate
- Erose
- Filamentous
- Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY / ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mcg	Te 30 mcg	
1-a						Shag
1-b						Shag/diplococci
1-c						Shag

E. ELEVATION

- Effuse
- Flat
- Raised
- Convex
- Umbonate

F. OPTICAL CHARACTERISTICS

- Opaque
- Translucent
- Opalescent
- Irridescent

G. HEMOLYSIS

- Positive
- Negative
- Alpha
- Beta

ND Not Done

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MICROBIAL IDENTIFICATION

CODE NUMBER: SH
 SUBJECT NAME: Stacy Hunt
 SITE: 2-Nose
 SWAB: MOIST - ✓ DRY -
 MEDIUM - NB
 DATE: 8/3/71 TIME: 1315

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ABSENT ✓

TAXO P - PRESENT ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
*Large, white colonies - smooth, shiny
 irreg. edge + large circular colony*

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G
2-a	G ⁺ cocci short chains	Neg	N-D	white	2	1	4	1	2
2-b	G ⁺ mycelium	Neg	N-D	white	2	6	3	1	2

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Commas
 - Spirals
 - Branched Rods
 - Mycelium
 - Chlamydospores
- C. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- D. EDGE
- Entire
 - Undulate
 - Lobate
 - Erose
 - Filamentous
 - Curled

B. COLOR

As Noted

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
2-a						Strep
2-b						Candida

E. ELEVATION

- Effuse
- Flat
- Raised
- Convex
- Umbonate

F. OPTICAL CHARACTERISTICS

- Opaque
- Translucent
- Opalescent
- Iridescent

G. HEMOLYSIS

- Positive
- Negative
- Alpha
- Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: SH
 SUBJECT NAME: Stacy Hunt
 SITE: 3 - forearm skin
 SWAB: MOIST - DRY -
 MEDIUM - NB
 DATE: 8/3/71 TIME: 1315

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION: growth to edge of disc
 TAXO A - PRESENT ABSENT
 TAXO P - PRESENT ABSENT
 NUMBER OF APPARENT COLONY TYPES: 1
 AROMA (IF PRESENT) NA
 DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Very sparse growth - small translucent colonies visible, few only

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.				COLONY CHARACTERISTICS			
		CATAL-ASE	CYTO-OXIDASE	B	C	D	E	F	G
3-a	G ⁺ cocci - <u>staphylococcus</u>								

DESCRIPTIVE KEYS:

- A. CELLULAR MORPHOLOGY
- Cocci
 - Diplococci
 - Rods
 - Commas
 - Spirals
 - Branched Rods
 - Mycelium
 - Chlamydozooids
- B. COLOR
- C. FORM
- Punctiform
 - Circular
 - Filamentous
 - Irregular
 - Rhizoid
 - Spindle
- D. EDGE
- Entire
 - Undulate
 - Lobate
 - Erode
 - Filamentous
 - Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER					PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	Te 30 mcg	
3-a						<u>Staph</u>

- E. ELEVATION
- Effuse
 - Flat
 - Raised
 - Convex
 - Umbonate
- F. OPTICAL CHARACTERISTICS
- Opaque
 - Translucent
 - Opalescent
 - Irridescent
- G. HEMOLYSIS
- Positive
 - Negative
 - Alpha
 - Beta

* Appearance indicated possibility of over decolorization - organisms were large coccid bodies in short chains, a possibility of G⁺ short rods.

6.5 COMMERCIAL PRODUCTS APPLICABLE TO THE I/D KEY